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# CEREAL CHEMISTRY

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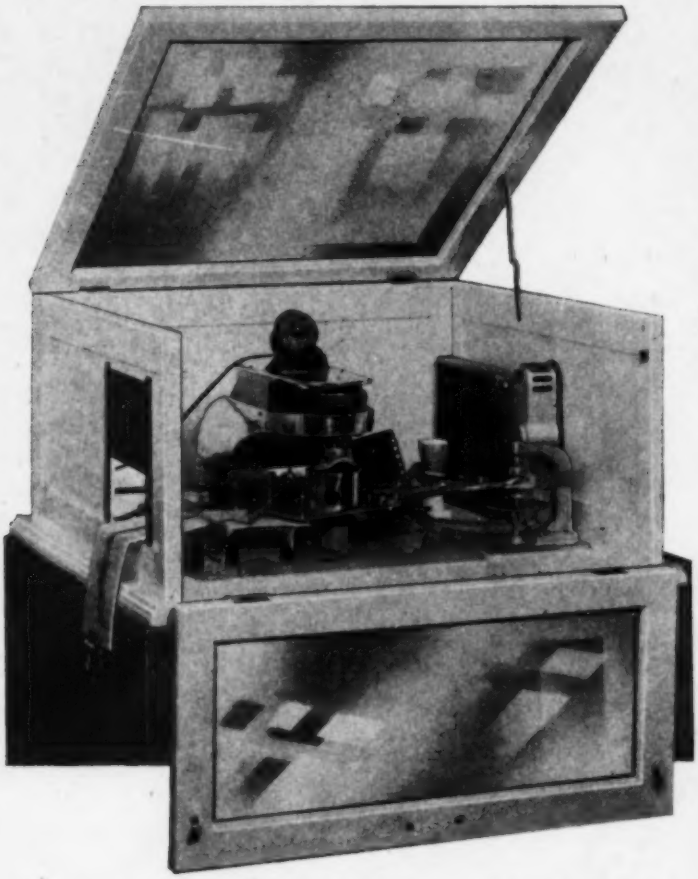

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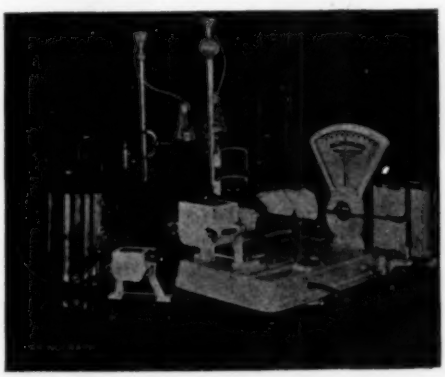

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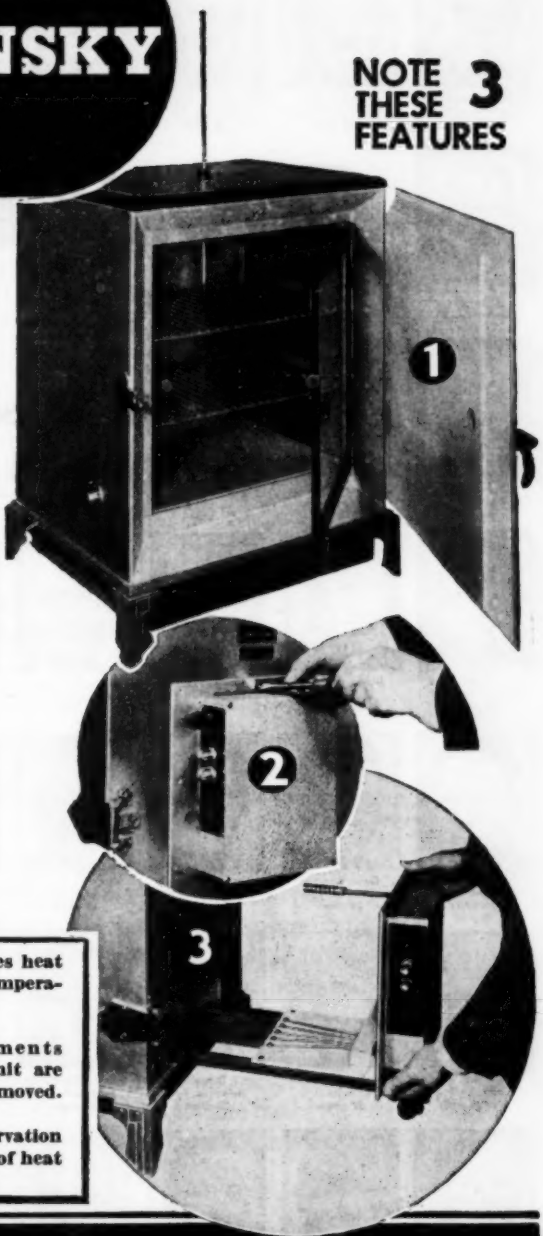


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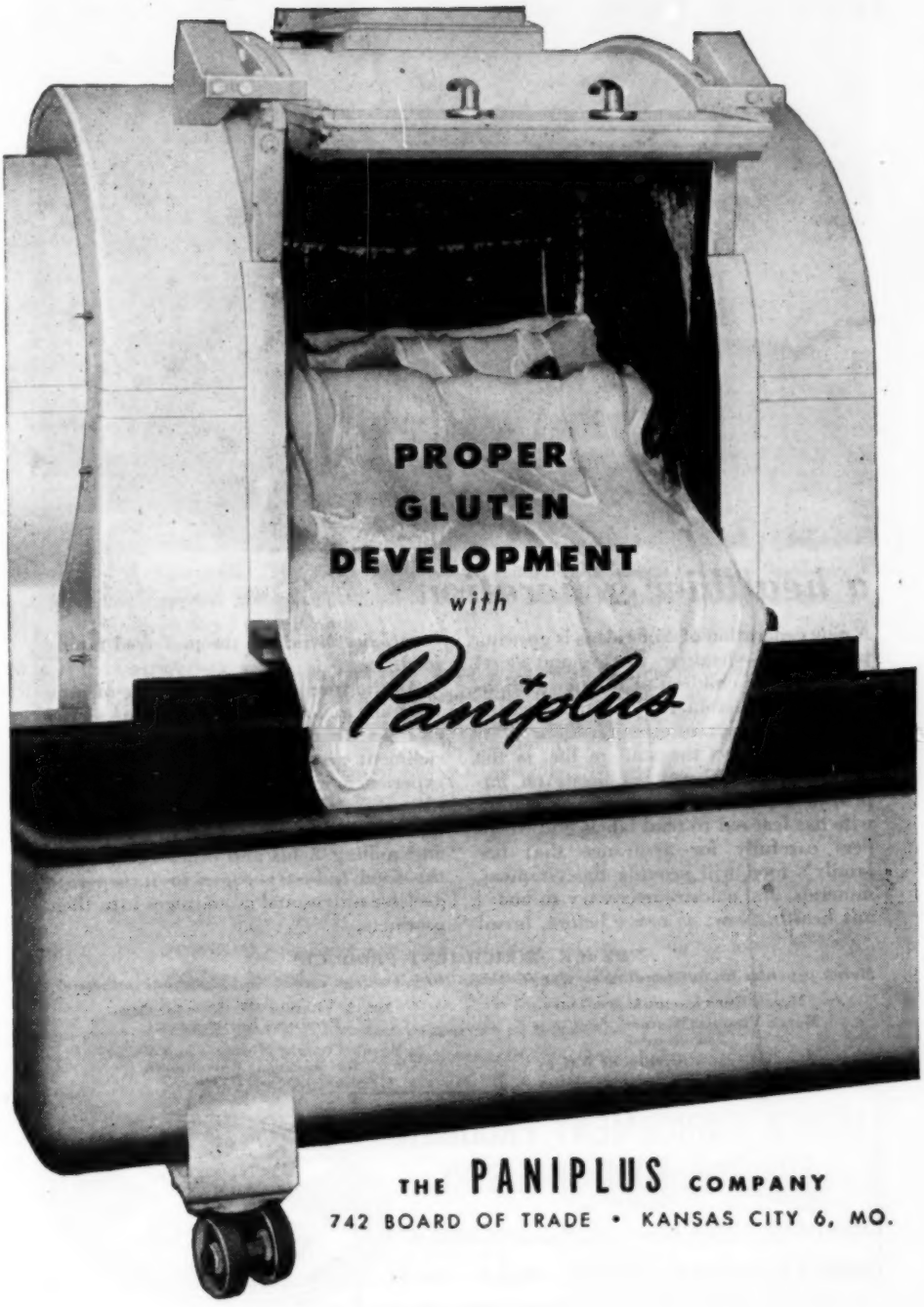


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CEREAL CHEMISTRY

VOL. XXV SEPTEMBER, 1948 No. 5

# LOAF VOLUME AND PROTEIN CONTENT OF HARD WINTER AND SPRING WHEATS [1]

KARL F. FINNEY and MARK A. BARMORE [2]

## ABSTRACT

Loaf volume data for samples covering a wide range in protein content are reported for hard winter and hard spring wheat varieties and for commercial samples representing four crops and grown under a wide range of climatic and soil conditions. A highly bromated, rich formula containing milk solids was employed in conjunction with optimum mixing time and water requirement.

The major factor accounting for variation in loaf volume within a variety was protein content, and the relation between theses two factors was essentially linear between the limits of protein encountered, i.e., 8 to 18%. Certain varieties, however, had distinctly different regression lines, the slopes of which, in general, increased as the loaf volume level became greater. The increase in loaf volume for each percentage increase in protein varied from about 40 cc. to 75 cc. for different varieties.

The loaf volume-protein content regression lines for varieties represent differences in protein quality. The loaf volume level for different varieties at 13.5% protein varied from 823 cc. to 1015 cc., a difference several times that required for statistical significance. Using the variety regression lines, a logical method of correcting loaf volumes for differences in protein content has been developed and used successfully for evaluating new and old varieties of hard winter and hard spring wheats.

Regression lines for loaf volume on protein content clearly demonstrate that the best quality hard winter and hard spring wheat varieties are equal in protein quality or breadmaking capacity as are also the medium and poor quality varieties of the two classes.

One of the principal tasks of the Hard Winter Wheat Quality Laboratory is to characterize experimental and commercial varieties of hard

[1] Manuscript received April 21, 1948.

The studies herein reported are a part of the cooperative work carried on by the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, and the agricultural experiment stations of the Great Plains Region, the Department of Milling Industry, Kansas Agricultural Experiment Station, and the Department of Agronomy, Ohio Agricultural Experiment Station.

[2] Chemists, Bureau of Plant Industry, Soils, and Agricultural Engineering, formerly stationed at the Hard Winter Wheat Quality Laboratory, Kansas Agricultural Experiment Station, Manhattan, Kansas, and the Federal Soft Wheat Laboratory, Ohio Agricultural Experiment Station, Wooster, Ohio; now at the State Agricultural Experiment Stations at Manhattan, Kansas, and Pullman, Washington, respectively.

red winter wheat so that they may be evaluated for use in the mill and bakeshop. The commercial suitability of new varieties is based on the similarity of their properties to those of the well-known commercial varieties. Because both hard winter and hard spring wheats are used primarily for bread flours, quality research on either class should be supplemented by a comparison with the other. Accordingly, representative hard spring wheat varieties have been included in the hard winter wheat research program since 1940.

One of the important properties that determines the suitability of a new variety of wheat for making bread is the relation of loaf volume to protein content. This paper presents the results of a four year study on the relation between protein content and loaf volume of bread for each of 14 hard winter and 8 hard spring wheat varieties. The relative loaf volume potentialities of hard red winter and hard red spring wheat flours are discussed together with the application of the data to the evaluation of new and experimental varieties of wheat. Subnormal loaf volumes, not accounted for by variety or protein content, are identified and classified according to flour mixing requirement.

## Review of Literature

Early work failed to show a linear relation between protein content and loaf volume. The data of Thomas (21), Shollenberger (20), and others indicated that with increasing protein content there was a diminishing increase in loaf volume up to 13 to 15% protein, depending on the class of wheat. Beyond 13 to 15% there was no increase in some instances and a sharp decline in others. Bailey and Sherwood (2), from data obtained on the 1921 to 1925 crops, inclusive, calculated the formula of the curve representing the relation between loaf volume and protein content. They found it to be hyperbolic, thus indicating that "each increment of increase in protein content results in a diminished increment of increase in loaf volume."

Thus, low correlation coefficients, many of which, however, were significant, were obtained previous to the work of Larmour (12) who obtained a correlation coefficient of 0.906 for loaf volume and protein content of Canadian hard red spring wheat grown in one season. Using composite samples representing the normal protein range, he concluded that the curvilinearity of the relation between protein content and loaf volume was limited to the extreme protein levels, and that the regression of loaf volume on protein was linear between the limits of 7% and 15.9%. Aitken and Geddes (1), using composite samples of Canadian spring wheat flour, found that the relation of loaf volume to protein content was approximately linear over the entire range of 11.4% to 15.7%.

Although most of the early work was carried out with comparatively lean baking formulas and small amounts of bromate, several recent publications describe baking methods employing a rich, highly bromated formula and approaching optimum conditions. All except Larmour (14) failed, however, to include optimum oxidation at the same time nonfat milk solids and shortening were present in the formula. Some did not use one or more of the following: shortening, nonfat milk solids, malt, or optimum mixing time. Finney and Barmore[3] (9) emphasized the importance of the combination of certain amounts of these ingredients and also showed (10) that the lack of proper mixing leads to erroneous conclusions for some varieties. The formula described by them and used with minor changes by a large number of workers gives loaf volumes which are an accurate expression of the quality and quantity of protein in the flour. With this type of formula, a linear relation between loaf volume and protein content has been reported by several workers.

Larmour, Working, and Ofelt (15), using composite samples of Turkey, Kanred, and Blackhull, representing one season, obtained a correlation between loaf volume and protein content of +0.98. Sandstedt and Ofelt (19) made a study of the comparative quality of certain wheat varieties at various naturally occurring protein levels representing one season. They found that the loaf volume and protein content were approximately linear within a variety. Similarly, the results of Bayfield, Working, and Harris (4), Bayfield and West (3), and Johnson, Swanson, and Bayfield (11) have shown that for a given season loaf volume and protein content were linear within a variety. Although correlation coefficients were not given, they were obviously high.

Finney (7), by means of a fractionating and reconstituting technique whereby the factors of soil and climate were eliminated, studied the relation between loaf volume and protein content over the range of about 0% to 20% protein, using varieties of wheat which differed in quality of protein. This work showed that loaf volume and protein content were linear between the limits of 7% or 8% to at least 20% protein. Below 7% protein, however, the relation was definitely curvilinear, all curves meeting at 0% protein and about 275 cc. loaf volume. The regression of loaf volume on protein content was different for different varieties and appeared to be a function of the loaf volume that was produced by a variety at any arbitrary protein level within the range of linearity. Regression lines for loaf volume on protein content suggested a fan-shaped family of lines that could be applied to determine the factor for correcting the loaf volume for a given sample to a constant protein basis from a knowledge of its protein content and loaf volume.

[3] A study of baking methods for evaluating the protein quality of hard winter wheats. Presented at annual AACC meeting, 1939.

Larmour (13) summarized the published information on hard spring and winter wheats and concluded that there was no evidence of fundamental difference in baking quality. Additional evidence in support of this view was obtained by Larmour (14).

## Materials and Methods

As part of the wheat improvement program carried out by the U.S. Department of Agriculture in cooperation with the state agricultural experiment stations, commercial and new varieties are grown throughout the hard winter and spring wheat regions for study. A complete description of the field studies is given by Quisenberry (17) and Clark (5). Grain of these varieties from carefully studied and widely different environments representing the hard wheat region was made available for milling and baking investigations. Thirty-one stations in 13 states covering the central United States from Texas to the Dakotas have supplied grain during at least one of the four years 1940–43, inclusive. Many have not been able to supply grain each year owing to poor conditions of one sort or another.

Nine samples of Thatcher grown in Saskatchewan and Alberta, Canada, in 1941 and 1942 were also included. A composite sample of commercially milled Thatcher flour was supplied each year by the Northwest Crop Improvement Association from its test plots in the spring wheat region.

The older varieties such as Nebred, Minturki, Tenmarq, Kharkof, Blackhull, Cheyenne, Yogo, Early Blackhull, Chiefkan, Pilot, Thatcher, Rival, and Marquis have all been described by Clark and Bayles (6). The newer varieties, Comanche and Pawnee, have been described by Reitz and Laude (18). Several of the newer varieties listed have been described previously only in mimeographed reports. Of these, Wichita has been recommended as a commercial variety in Oklahoma, Texas, and Kansas; Red Chief is a new wheat produced by Earl Clark, a private breeder of Sedgwick, Kansas; Merit and Premier are two new spring wheats that were included in the earlier studies but probably will not be released for commercial production.

For the study of the relation of milling and baking characteristics to market grade of hard winter wheat, the Federal Grain supervisors of the Grain Branch, Production and Marketing Administration, U. S. Department of Agriculture, furnished composite samples of the major market grades collected at the principal terminal markets in the winter wheat region during each of three crop years.

All samples except Thatcher from the Northwest Crop Improvement Association were obtained as wheat and milled experimentally on a Buhler or Allis mill. The unbleached flours were stored at about 70°F.

for 3 weeks and then at about 40°F. for one or more months until baked. The baking method involved the use of optimum mixing time, and the following rich, highly bromated formula: flour 100 g., water as needed, sugar 6 g., salt 1.5 g., shortening 3 g., yeast 2 g. (standardized), nonfat milk solids 4 g., 120°L. malt syrup 0.25 g., and potassium bromate 1 to 5 mg. The loaf volume for a variety was that for the optimum amount of bromate. The doughs were fermented for 3 hours and proofed for 55 minutes at 86°F. The baking method is described in more detail by Finney and Barmore (9 and 10).

Differences in loaf volume of 20 to 25 cc. are required for significance when using the same lot of yeast. When using a given lot of flour, but using different shipments of yeast, 30 to 35 cc. differences are required for significance, even when the largest variations in yeast are eliminated according to the procedure of Finney and Barmore (8). Another error of about 25 to 35 cc. may be introduced due to the effect of slight variations in experimental milling on loaf volume. Thus it would be possible for these different errors to be all positive or negative on any one sample, thereby accounting for occasional loaf volume fluctuations about a varietal regression line of as much as 70 cc.

## Results

The relation between loaf volume and protein content for the hard winter varieties is shown graphically in Figs. 1 and 2 and for the hard springs in Fig. 3. A letter has been used to identify the source of each sample. The lines shown are the regression lines, the equations for which are given in Table I along with other pertinent statistical data.

*Hard Winter Wheat Varieties.* Certain hard winter samples tested during the four year period reported here were regarded as subnormal in mixing time, dough-handling properties, and loaf volume and unsatisfactory for quality evaluation, just as are samples with extremely low test weights. Since the subnormal loaf volumes are definitely accounted for by factors other than variety and protein content, statistical calculations were made both including and excluding these subnormal samples. Subnormal samples are identified in Figs. 1 and 2 and the data and discussion pertaining to them are presented later. The correlation coefficients given in Figs. 1 and 2 were obtained when the subnormal samples were omitted.

The relation between loaf volume and protein content is essentially linear within a variety (Figs. 1 and 2). Considering the large number of individual samples representing several crop years, and the extremes in environment for each variety, the correlation coefficients are more highly significant than those obtained by previous investigators. These data show that protein content accounts for nearly 90% of the varia-

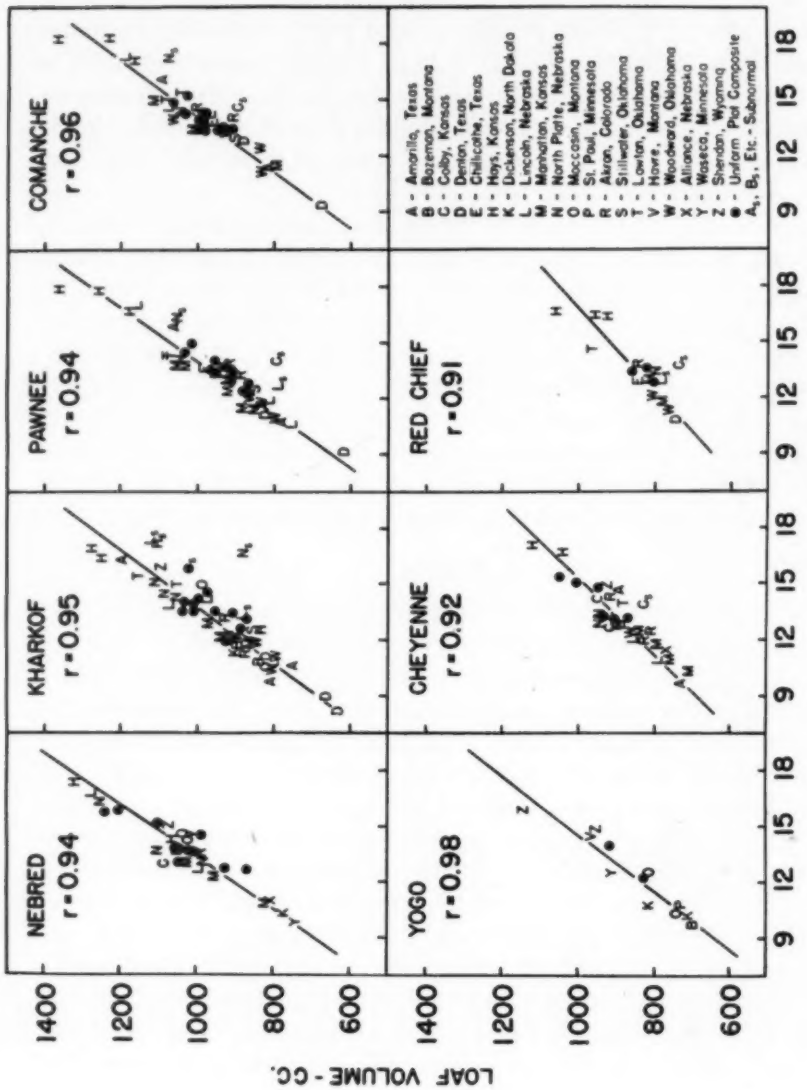


Fig. 1. Loaf volume and protein content for seven hard winter wheat varieties grown for several seasons under widely different conditions of climate and soil. Key identifies each sample as to source.

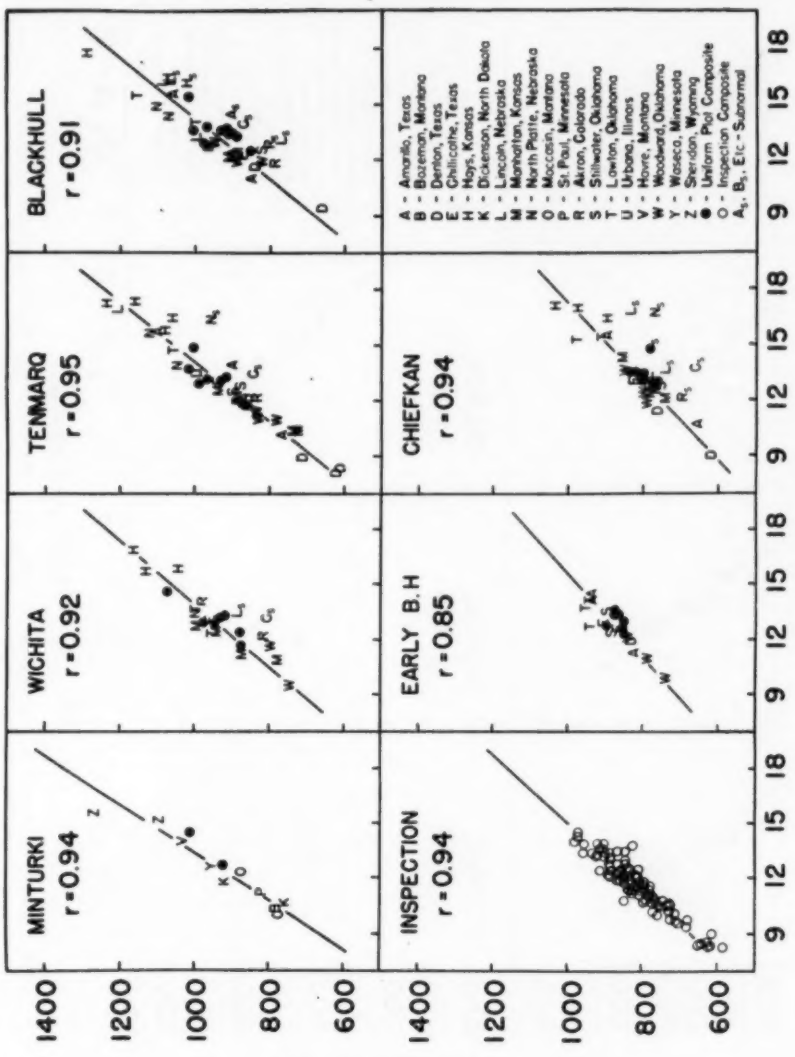


Fig. 2. Loaf volume and protein content for inspection samples and six hard winter wheat varieties grown for several seasons under widely different conditions of climate and soil. Key identifies each sample as to source.

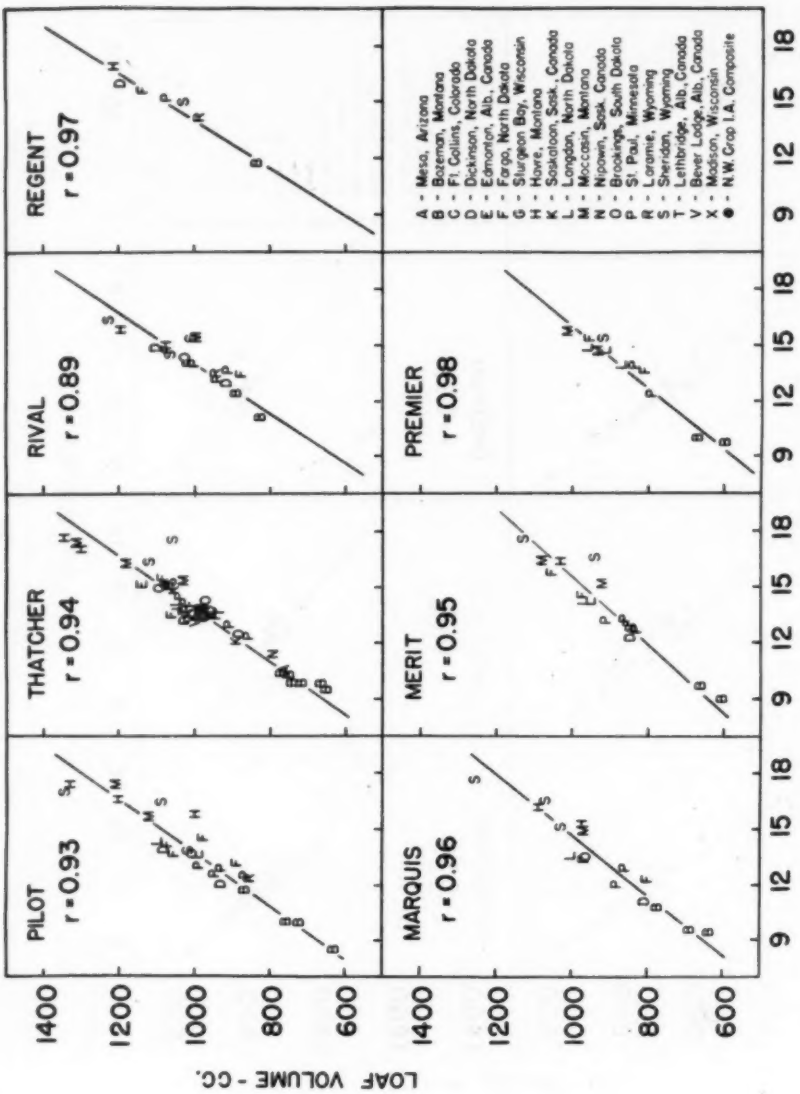


Fig. 3. Loaf volume and protein content for seven hard spring wheat varieties grown for several seasons under widely different conditions of climate and soil. Key identifies each sample as to source.

TABLE I

STATISTICAL SUMMARY OF PROTEIN CONTENT AND LOAF VOLUME DATA FOR HARD WINTER AND HARD SPRING WHEAT VARIETIES

| Variety | C.I. number | Crop years (incl.) | No. samples | | Corr. coeff. ($r$) | | Regression equation[1] | Mean flour protein[2] | Mean loaf volume[2] | Loaf volume at[3] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Without subnormal samples | All samples | Without subnormal samples | All samples | | | | 13.5% protein | 16% protein |
| | | | | | | | | % | *cc.* | *cc.* | *cc.* |
| **Hard Winter Varieties** | | | | | | | | | | | |
| Nebred | 10094 | 1940–42 | 30 | 30 | 0.94 | 0.94 | 73.15X+ 30.6 | 13.6 | 1029 | 1018 | 1201 |
| Minturki | 6155 | 1940–42 | 12 | 12 | .95 | .95 | 75.19X− 8.4 | 12.5 | 935 | 1007 | 1211 |
| Kharkof | 1442 | 1940–43 | 47 | 52 | .95 | .89 | 65.33X+102.6 | 12.8 | 938 | 985 | 1148 |
| Wichita | 11952 | 1940–43 | 22 | 24 | .92 | .87 | 58.86X+177.7 | 13.0 | 943 | 972 | 1120 |
| Pawnee | 11669 | 1940–43 | 39 | 42 | .95 | .92 | 70.94X+ 8.9 | 13.3 | 953 | 967 | 1142 |
| Tenmarq | 6936 | 1940–43 | 38 | 40 | .95 | .92 | 61.76X+129.2 | 12.9 | 925 | 963 | 1117 |
| Comanche | 11673 | 1940–43 | 36 | 38 | .96 | .95 | 67.13X+ 55.0 | 14.0 | 997 | 961 | 1129 |
| Blackhull | 6251 | 1940–43 | 33 | 39 | .91 | .87 | 61.23X+127.2 | 13.5 | 954 | 954 | 1107 |
| Yogo | 8033 | 1940–42 | 12 | 12 | .95 | .95 | 62.36X+ 88.5 | 12.3 | 854 | 930 | 1086 |
| Inspection | — | 1940–42 | 114 | 114 | .94 | .94 | 54.41X+181.9 | 11.6 | 814 | 917 | 1053 |
| Cheyenne | 8885 | 1940–43 | 30 | 31 | .92 | .90 | 49.78X+243.6 | 13.1 | 894 | 916 | 1040 |
| E. Blackhull | 8856 | 1940–43 | 21 | 21 | .85 | .85 | 43.76X+317.3 | 12.8 | 875 | 908 | 1018 |
| Red Chief | 12109 | 1942–43 | 15 | 17 | .91 | .85 | 43.90X+261.1 | 13.5 | 854 | 854 | 964 |
| Chiefkan | 11754 | 1940–43 | 27 | 33 | .94 | .76 | 46.94X+189.6 | 13.4 | 819 | 823 | 941 |
| **Hard Spring Varieties** | | | | | | | | | | | |
| Pilot | 11945 | 1940–43 | — | 26 | — | 0.93 | 68.99X+ 55.3 | 13.7 | 1002 | 987 | 1159 |
| Thatcher | 10003 | 1940–43 | — | 49 | — | .94 | 70.10X+ 30.5 | 13.6 | 989 | 977 | 1152 |
| Rival | 11708 | 1941–43 | — | 18 | — | .89 | 74.05X− 42.3 | 14.2 | 1008 | 958 | 1143 |
| Regent | 12070 | 1943 | — | 7 | — | .97 | 78.56X−107.9 | 15.0 | 1068 | 953 | 1149 |
| Marquis | 3641 | 1940–42 | — | 16 | — | .96 | 61.24X+105.6 | 13.4 | 925 | 932 | 1085 |
| Merit | 11870 | 1940–42 | — | 17 | — | .95 | 54.41X+155.4 | 13.9 | 914 | 890 | 1026 |
| Premier | 11940 | 1940–41 | — | 12 | — | .98 | 60.30X+ 32.4 | 13.7 | 859 | 846 | 997 |
| Progress | 6902 | 1940–41 | — | 4 | — | .99 | 40.50X+293.5 | 13.5 | 838 | 840 | 942 |

[1] Loaf volume vs. flour protein (X) without subnormal samples (all hard spring data used).
[2] Omitting samples with critically subnormal mixing times (all hard spring data used).
[3] Calculated from regression equation.

tions in loaf volume within a variety irrespective of the season and such environmental factors as soil and climate, if the samples identified by critically subnormal mixing times are excluded.

The differences between the regression lines (subnormal samples omitted) for the hard winter wheat varieties are strikingly illustrated in Fig. 4 in which the regression lines for all varieties are shown. The re-

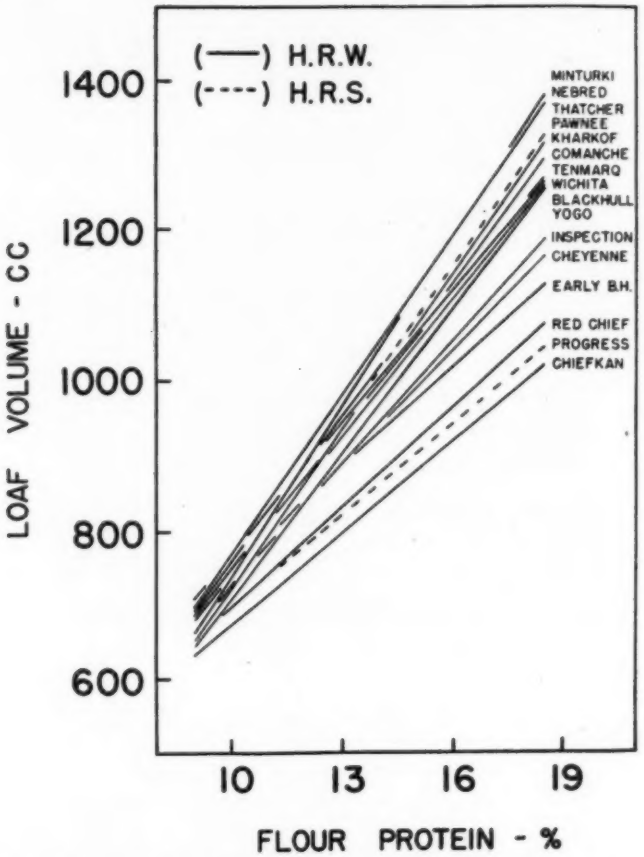


Fig. 4. Loaf volume-protein content regression lines for 13 hard winter wheat varieties, the inspection composites, and two hard spring wheat varieties.

gression lines for two varieties of spring wheat, Thatcher and Progress, are included for comparison. The data show that, in general, the regression coefficients or slopes of the regression lines increase as loaf volume level increases. Thus the increase in loaf volume for each per cent increase in protein varies from 46.9 cc. for Chiefkan, which has the smallest loaf volume, to 73.2 and 75.2 cc. for Nebred and Minturki, respectively, the two varieties that gave the largest loaf volumes. This "fanning-out" of the regression lines is shown to be real

by the very highly significant correlation coefficient $r = 0.94$, obtained when their slopes were compared with the corresponding loaf volumes at 16% protein. When the slopes for the regression lines for all samples were correlated with the corresponding loaf volumes at 16% protein, an equally significant value $r = 0.95$, was obtained.

By analysis of covariance the slopes of the variety regression lines for all samples were found to be heterogeneous ($P \ll 0.001$). This fact justified the testing for significance of various pairs of slopes within the group of 14 regression lines, and it was found that the slope of Chiefkan differed significantly from those for Nebred, Pawnee, Comanche, and the Inspection samples ($P \lessapprox 0.05$). Other pairs of slopes that differed statistically include Early Blackhull with Nebred and Minturki; Cheyenne with Nebred; and Inspection with Minturki, Nebred, and Pawnee ($P \lessapprox 0.05$). Several other pairs of slopes approached statistical significance. When the subnormal samples were omitted in a similar analysis, the slopes for the group of regression lines likewise were found to be heterogeneous ($P \ll 0.001$). The slopes of Chiefkan, Early Blackhull, and Inspection differed significantly from the slopes of Minturki, Nebred, Pawnee, and Comanche. Likewise, the slopes for Cheyenne and Nebred and for Early Blackhull and Kharkof differed significantly ($P \lessapprox 0.05$). In addition, the differences between the slopes for numerous other pairs of varieties approached significance.

The loaf volumes for certain varieties are considerably different, especially at the high protein levels. At 13.5% protein, for example, the loaf volume for Chiefkan is 823 cc. and for Nebred 1018 cc., a difference of nearly 200 cc. At 16% protein, however, the difference between Nebred and Chiefkan is about 260 cc. Thus greater differentiation is obtained at the higher protein levels. Each of the more important loaf volume differences at 16% protein was compared with the corresponding standard error of the difference. Since the sampling variation of the regression coefficients was ignored, larger values for "t" at the 5%, 1%, and 0.1% level of significance were used than would have been otherwise necessary. For example, a value for "t" ordinarily regarded as significant at the 1% point was interpreted as indicating significance at the 5% point. The difference of 59 cc. between Nebred and Pawnee at 16% protein was significant at the 1% point but was interpreted as being significant only at the 5% point. In general a loaf volume difference of 50 to 60 cc. between two varieties was interpreted as being significant at the 5% point; whereas a difference of about 100 cc. was significant even beyond the 0.1% level. The loaf volume difference of 260 cc. between Nebred and Chiefkan was 13.9 times the standard error of the difference and accordingly was significant far beyond the 0.1% level. Out of 64 loaf volume differences at 16% protein that were

statistically significant ($P \lessapprox 0.05$), 44 were significant at $P \lessapprox 0.001$. Even when the subnormal samples were included, 56 loaf volume differences between various pairs of varieties were statistically significant ($P \lessapprox 0.05$), 33 of which were significant at $P \lessapprox 0.001$.

The ranking of this group of hard winter wheat varieties by their regression lines corresponds with their generally recognized loaf volume potentialities. Accordingly, loaf volumes on a constant protein basis can be used as a reliable index of relative breadmaking capacity or protein quality. For a comparison of loaf volumes not on a comparable protein basis, these variety regression lines can be used to correct loaf volume to a constant protein basis. About 13.5% protein is the average of all samples encountered and thus should be the most satisfactory constant basis for keeping the amount of correction to a minimum.

Loaf volume on a constant protein basis (which is regarded as a measure of protein quality), however, is by no means the only property which determines the suitability of a variety for bread-baking purposes. Although probably the most important, it must be considered in conjunction with other important properties such as wheat hardness and milling quality, dough mixing, oxidation, and water requirements, and bread crumb grain and color scores.

*Hard Spring Wheat Varieties.* The relation between loaf volume and protein content for the spring wheat flours, presented in Fig. 3, is also essentially linear within a variety. The correlation coefficients show that protein content accounts for fully 90% of the variations in loaf volume within a variety, or slightly more than for hard winter wheat regardless of the season, soil, and climate.

The differences between the regression lines for loaf volume on protein content for the hard spring wheat varieties are illustrated in Fig. 5, which also includes regression lines for two hard winter varieties for comparison. As was true for the hard winter wheats, the slopes of the regression lines, in general, increase as loaf volume increases. Referring to Table I, the increase in loaf volume for each per cent increase in protein varies form 40 cc. for Progress, which has the smallest loaf volume, to 74 cc. for Rival and 78 cc. for Regent, two varieties possessing superior loaf volume potentialities. Regent, however, is represented by only one crop year. This "fanning-out" of the regression lines is shown to be real by the very highly significant $r = 0.92$, obtained when their slopes were compared with the corresponding loaf volumes at 16% protein.

Analysis of covariance indicated that the slopes for the various hard red spring regression lines were not heterogeneous. Hence it appears that the "fanning-out" of the regression lines is not sufficiently pronounced to be statistically significant in terms of a test for nonhomo-

geneity of the sampling slopes. The hard spring wheat samples generally were smaller in number than those for the hard winter wheats. That fact probably has contributed to the failure to find statistically significant heterogeneity of the slopes of the regression lines in spite of a very highly significant correlation of 0.92 between slope and loaf volume at 16% protein.

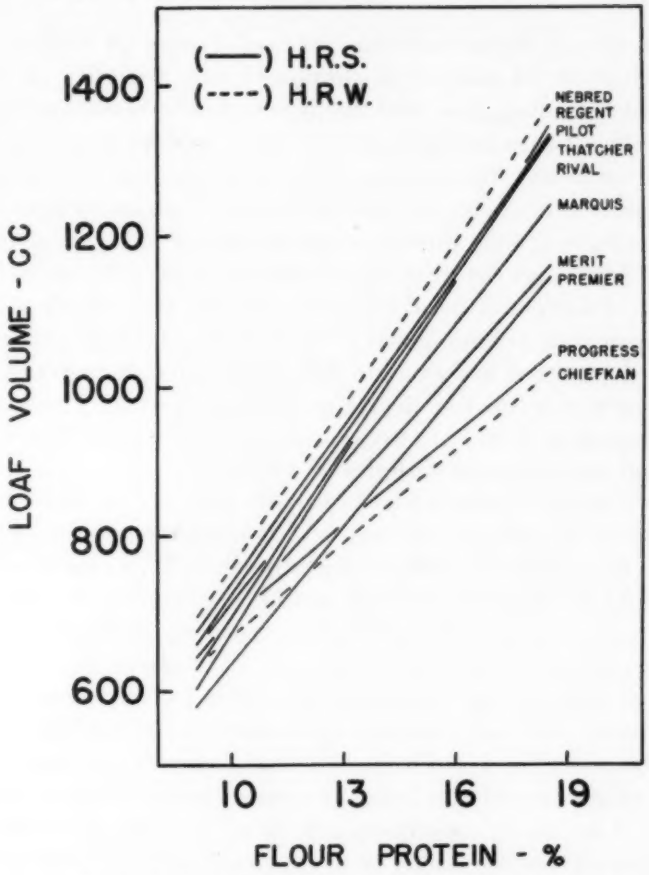


Fig. 5. Loaf volume-protein content regression lines for eight hard spring and two hard winter wheat varieties.

Large loaf volume differences exist between certain hard spring varieties at the higher protein levels. For example, at 16% protein the loaf volume for Progress is only about 940 cc. in contrast to about 1150 cc. for Pilot, Thatcher, Regent, and Rival. Each of the more important loaf volume differences at 16% protein was compared with the corresponding standard error of the difference. In general, a loaf volume difference of 40 to 50 cc. between two varieties was significant at the 5% point; whereas a difference of about 90 cc. was significant at or be-

yond the 0.1% level. The loaf volume difference of 162 cc, between Thatcher and Premier was 9.7 times the standard error of the difference for these two varieties and accordingly was singificant far beyond the 0.1% level. Out of 21 loaf volume differences at 16% protein that were statistically significant ($P \lessapprox 0.05$), 16 were significant at $P \lessapprox 0.001$.

## Discussion

*Application of Regression Lines for Loaf Volume on Protein Content.* The relatively small number of samples of some varieties, particularly of the hard red spring class, and the high variances of estimate of others make it difficult to establish some differences in slope that probably are real. Nevertheless, the heterogeneity of the slopes for the hard red winters taken as a whole, the very high correlation coefficients for slope and loaf volume at 16% protein for both winters and springs, the significance of some and the near significance of other differences between the slopes of various pairs of varieties, and the very highly significant differences in loaf volume levels with high protein content leave little room for doubt that loaf volume differs for different varieties and for different protein levels to a degree and in such a manner as to warrant the application of these data to experimental baking and the evaluation of new and experimental varieties of wheat.

Protein content of hard wheats usually varies from about 8 to 20% depending on the climate and soil. Varieties grown presumably under identical conditions have differed by as much as 2% in protein content, which might account for as much as 150 cc. difference in loaf volume. In order to measure protein quality it is necessary that these variations in protein content be taken into account. The obvious way to do this is to adjust loaf volumes to a constant protein level by means of the regression lines. It is of fundamental importance to note that if the regression lines of Figs. 4 and 5 are extended they tend to pass through a common origin within the limits of experimental error at about 7% protein. A necessary consequence of this is that the regression lines of all varieties represent a family of lines of varying slopes which increase with loaf volume at any given protein level. By making use of this fact, it is possible to construct a correction chart showing the regression lines for all possible varieties regardless of protein quality. Such a graph is is shown in Fig. 6.

Obviously, the value of the application of Fig. 6 lies in the ability to compare the relative protein quality of new and of established varieties on a constant protein basis. Since loaf volume for a given protein content is a characteristic of the variety and determines its regression line, the loaf volume and protein content of a flour of unknown variety can be used to identify that variety as regards its general protein-quality

class. For example, flour from an unknown variety has a protein content of 15% and gives a loaf volume of 900 cc. The point on Fig. 6 determined by this protein content and loaf volume is located on the regression line whose slope is 45. Following down this line to 13.5% protein gives a loaf volume of about 835 cc. which, according to Fig. 4, is midway between the loaf volumes of Red Chief and Chiefkan at 13.5% protein. The unknown flour is now described as having poor pro-

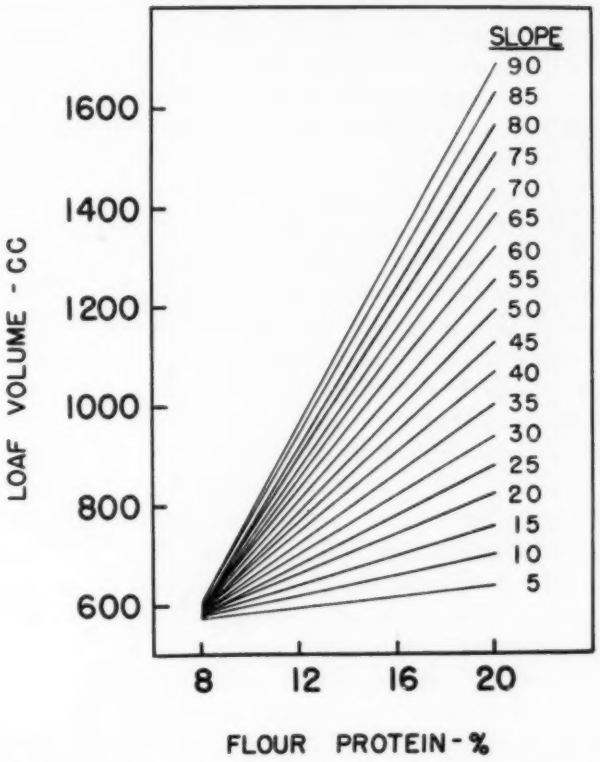


Fig. 6. Chart for correcting loaf volume to a constant protein basis.

tein quality about equal to that of Red Chief or Chiefkan. The protein level of 13.5% used for comparison in the above example is about the average of all samples tested. When correcting the loaf volumes of any particular group of samples, however, it is desirable to use their mean protein content as the constant protein basis, and thereby apply a minimum of correction to the group as a whole.

The application of this procedure of correcting loaf volumes for differences in protein content is not without error and especially so when correcting the loaf volumes of extremely low protein samples to a relatively high protein basis such as 13.5%. Nevertheless, it is derived

logically from the data at hand and is more accurate than a correction factor based on an average of the regression lines of many varieties.

This method of correcting loaf volumes should not be applied to results obtained by baking methods which are not optimum or are lacking in some important ingredient. It might apply, however, to other formulas if it can be shown that the location and slopes of the regression lines are similar to those shown in Figs. 4, 5, or 6. For example, the regression lines reported by Larmour (14) fit very well, as do those of Johnson, Swanson, and Bayfield (11) when using a similar formula. The results of Larmour (14) and McCalla (16) obtained by means of the malt-phosphate-bromate formula, however, are not in agreement. It is quite possible that the goodness of fit of other regression lines to the general family of lines in Fig. 6 may be a measure of whether or not the baking method used was optimum.

*Comparison of Hard Winter and Hard Spring Wheat Varieties.* This study of hard winter and hard spring wheat varieties for several seasons makes possible an accurate comparison of the protein quality of the two classes of wheat. The regression lines for loaf volume on protein content of two hard spring wheat varieties have been included with those for the hard winters of Fig. 4; and those for two winters have been included with the regression lines for the hard springs of Fig. 5. An examination of those figures and the loaf volume data at 13.5% protein given in Table I shows that the loaf volume potentialities of representative hard spring wheat varieties covers about the same range as that of hard winter wheats. For example, Thatcher and Pilot appear to be very nearly equal to Nebred and Minturki and slightly better than Pawnee and Comanche; Marquis is equal to Blackhull and slightly below Kharkof, Wichita, and Tenmarq; Merit is similar to Cheyenne, whereas Premier is somewhat poorer than Early Blackhull; and Progress is about midway between the two poorest hard winter wheats, Red Chief and Chiefkan. Larmour (13 and 14) came to this same general conclusion after summarizing the published information on hard winter and spring wheats and conducting baking tests on one crop of both classes of wheat. In determining the suitability of a wheat variety for breadmaking purposes, as has been previously pointed out, the property of loaf volume-producing ability at a given protein level (regarded as an index of protein quality) must be considered in conjunction with other important properties such as wheat hardness and milling quality; dough mixing, oxidation, and water requirements; and bread crumb grain and color scores.

*Subnormal Samples.* Occasionally the flours milled from wheats grown at certain stations have been characterized by subnormal mixing requirements. When the degree of subnormality reaches a certain

critical point, two significant facts have been noted in connection with such samples, namely, the dough-handling properties are comparatively poor and the loaf volumes are always considerably below normal.

Recent studies to be presented in a later publication indicate that these subnormal mixing times and loaf volumes are a function of high temperatures during the fruiting period. Thus, in studying the relation

TABLE II

LOAF VOLUME FOR VARIETY SAMPLES CHARACTERIZED BY SUBNORMAL MIXING TIMES, MANY OF WHICH ARE CRITICALLY SUBNORMAL

| Variety | Mixing time | | Loaf volume | | Mixing time | | Loaf volume | |
|---|---|---|---|---|---|---|---|---|
| | As rec. | Expected[1] | As rec. | Expected[2] | As rec. | Expected[1] | As rec. | Expected[2] |
| | *min.* | *min.* | *cc.* | *cc.* | *min.* | *min.* | *cc.* | *cc.* |
| | NORTH PLATTE—1940 | | | | AKRON—1942 | | | |
| Kharkof | 1⅛ | 2¼ | *881*[3] | 1160 | 1⅝ | 2¼ | 928 | 950 |
| Blackhull | 1⅜ | 2 | *1072* | 1123 | 1½ | 2 | *805* | 910 |
| Tenmarq | 1⅛ | 3¼ | *958* | 1145 | 2¾ | 3¼ | 888 | 875 |
| Cheyenne | — | — | —[4] | — | 3¼ | 4⅜ | 845 | 845 |
| Nebred | 2¼ | 3⅛ | 1263 | 1230 | 2¼ | 3⅛ | 1003 | 1025 |
| Pawnee | 1½ | 2⅛ | *1055* | 1165 | 1¾ | 2⅛ | 895 | 945 |
| Comanche | 1½ | 3⅛ | *1075* | 1210 | 2⅛ | 3⅛ | 1010 | 1028 |
| Chiefkan | 1⅛ | 2⅛ | *768* | 942 | 1½ | 2⅛ | *698* | 758 |
| Wichita | — | — | —[4] | — | 2 | 2¾ | 978 | 1000 |
| | COLBY—1943 | | | | LINCOLN—1943 | | | |
| Kharkof | — | — | —[4] | — | 1⅜ | 2¼ | *885* | 955 |
| Blackhull | 1¼ | 2 | *868* | 970 | 1¼ | 2 | *765* | 915 |
| Tenmarq | 2 | 3¼ | *843* | 957 | 2½ | 3¼ | 888 | 903 |
| Cheyenne | 3 | 4⅜ | *827* | 935 | 3¼ | 4⅜ | 852 | 845 |
| Pawnee | 1⅝ | 2⅛ | *795* | 950 | 1½ | 2⅛ | *790* | 880 |
| Comanche | 1⅝ | 3⅛ | 893 | 1035 | 1⅞ | 3⅛ | 960 | 995 |
| Chiefkan | 1¼ | 2⅛ | *658* | 800 | 1⅛ | 2⅛ | *738* | 790 |
| Wichita | 1⅜ | 2¾ | *805* | 950 | 1⅛ | 2¾ | *882* | 965 |
| Chey. x Tq. | 1⅝ | 3⅜ | *788* | 900 | 2¼ | 3⅜ | 885 | 865 |
| Red Chief | 1½ | 2⅜ | *735* | 855 | 1⅜ | 2⅜ | *778* | 825 |

[1] Average of several years data.
[2] Expected on basis of protein content of samples and variety regression line.
[3] Definitely subnormal loaf volumes are italicized.
[4] Not available for testing.

of loaf volume to protein content within and between varieties, it appears imperative that the effect of high temperature during the fruiting period on mixing time and loaf volume be considered. This has been accomplished to a limited degree in the work reported here by considering separately all wheat samples which are characterized by critically subnormal mixing times.

Practically all samples that have been characterized as having seriously subnormal mixing times during the crop years reported in this paper were grown at North Platte, Nebraska, in 1940, Colby, Kansas, in 1943, and Lincoln, Nebraska, in 1943. Samples representing an appreciably less degree of mixing time subnormality than those just mentioned were grown at Akron, Colorado, in 1942. The mixing times and loaf volumes, both as received and expected, for the samples from these four stations are shown in Table II. The results for a number of these samples, most of which are from Akron, are not seriously subnormal but are included to complete the picture indicating the degree of subnormality of mixing time associated with subnormal loaf volumes. The loaf volumes considered subnormal are italicized.

Out of the total of 505 hard winter wheat samples tested, there were no others which were characterized by the extreme degree of mixing time and loaf volume subnormality typical of many of the samples in Table II. There were, however, several others whose mixing times either approached or were border-line samples of critical subnormality or whose loaf volumes were sufficently subnormal to warrant attention. The mixing times and loaf volumes for these samples are given in Table III.

TABLE III

OTHER SAMPLES WITH MIXING TIME OR LOAF VOLUME SUFFICIENTLY SUBNORMAL TO WARRANT ATTENTION (ONLY OTHERS IN ADDITION TO THOSE GIVEN IN TABLE II)

| Variety | Station | Year | Mixing time | | Loaf volume | |
|---|---|---|---|---|---|---|
| | | | As rec. | Expected[1] | As rec. | Expected[2] |
| | | | *min.* | *min.* | *cc.* | *cc.* |
| Kharkof | Lincoln, Neb. | 1940 | 1⅝ | 2¼ | *1122*[3] | 1220 |
| Kharkof | Lincoln, Neb. | 1941 | 1½ | 2¼ | 1066 | 1015 |
| Kharkof | Hays, Kans. | 1943 | 1¾ | 2¼ | *1120* | 1220 |
| Blackhull | Hays, Kans. | 1940 | 1⅝ | 2 | *1022* | 1125 |
| Blackhull | Lincoln, Neb. | 1940 | 1½ | 2 | *1078* | 1125 |
| Blackhull | Amarillo, Tex. | 1942 | 1¾ | 2 | *900* | 1005 |
| Yogo | Waseca, Minn. | 1942 | 1½ | 2 | 915 | 865 |
| Early B. H. | Amarillo, Tex. | 1943 | 1½ | 2 | 933 | 948 |
| Chiefkan | Lincoln, Neb. | 1940 | 1⅞ | 2⅛ | *830* | 960 |
| Chiefkan | North Platte, Neb. | 1942 | 1⅜ | 2⅛ | *748* | 800 |

[1] Average of several years data.
[2] Expected on basis of protein content of sample and variety regression line.
[3] Loaf volumes considered subnormal are italicized.

The mixing times for the samples from North Platte and Colby (Table II) are definitely subnormal compared with those expected from an average of several years data. In addition, all loaf volumes are subnormal except that for Nebred from North Platte. Similarly the mixing times for the samples from Lincoln are subnormal but to a somewhat

less extent for most samples than are those from Colby and North Platte. This is also true for loaf volume. Although the mixing times for all samples from Akron are subnormal, they are only slightly so, and only two loaf volumes are subnormal.

A clearer picture of the extent to which mixing time may be subnormal without resulting in subnormal loaf volume is shown in Fig. 7.

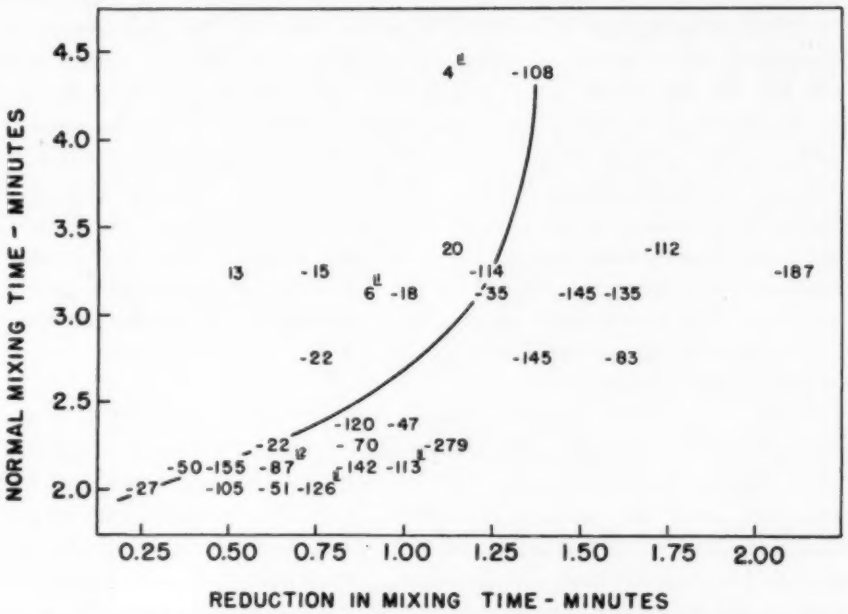


Fig. 7. Loaf volume deviations from normal when mixing times are subnormal (a) by varying amounts, normal mixing time being held constant along the horizontal and (b) by fixed amounts, normal mixing time being increased upward along the vertical. ($\underline{1}$ and $\underline{2}$—average of two and three determinations, respectively).

When inspecting the loaf volume deviations along the horizontal of Fig. 7, it will be noted that a reduction in normal mixing time of ½ minute to 1⅛ minutes for normal mixing times of 3⅛ to 3⅜ minutes does not result in loaf volume reductions (Table II). For a reduction greater than 1⅛ minutes, however, large loaf volume reductions follow. Thus 1⅛ to 1¼ minutes is considered to be the *critical reduction* for a normal mixing time of 3⅛ to 3⅜ minutes. As normal mixing time decreases, the *critical reduction* value decreases until at a normal mixing time of 2 minutes it is only about ¼ minute, since all mixing times that were below normal by more than ¼ minute are associated with loaf volumes that are materially *below* normal. Instead of working along the horizontal, consider the loaf volume deviations on the vertical at fixed reductions in mixing time. For example, a reduction of ½ minute for normal mixing times of 2 and 2⅛ minutes

resulted in loaf volume reductions of 105 cc. and 155 cc. When the same reduction in mixing time of ½ minute was associated with a normal mixing time of 3¼ minutes, however, there was no reduction in loaf volume. Similarly, a reduction in mixing time of ⅞ minute for a normal mixing time of 2⅛ to 2⅜ minutes is associated with an average loaf volume reduction of over 100 cc., but for a longer normal mixing time of 3⅛ minutes does not result in a loaf volume reduction.

The curve in Fig. 7 has been drawn so that all loaf volume deviations accounted for by experimental error are appreciably above or to the left of the curve; whereas all those representing varying degrees of significance are below or to the right. This curve can be used to estimate the critical mixing times for known varieties of wheat having different normal mixing times. Thus for variety samples that normally have mixing times of from 2 to about 2½ minutes, 1⅝ (2¼–⅝) is the critical mixing time below which loaf volume likely will be subnormal. For mixing times above 2½ minutes, however, this critical point increases as normal mixing time increases. Thus mixing times of about 2 minutes for Tenmarq and 3 minutes for Cheyenne represent critical points.

The only other samples which were characterized by either a marked subnormal loaf volume or mixing time approaching or equal to that considered as critical are given with their mixing times and loaf volumes, both as received and expected, in Table III. The mixing times for 6 of the 10 samples given in Table III have mixing times of 1½ to 1⅝ minutes and are therefore border-line cases with respect to being critical. Thus the corresponding loaf volume could be either normal or subnormal without introducing any inconsistencies. Of these six samples, the loaf volumes for Kharkof and Blackhull from Lincoln, Nebraska, in 1940, and Blackhull from Hays, Kansas, in 1940, are probably definitely subnormal on the basis of the errors attributed to baking techniques, yeast variability, and experimental milling. Similarly, the loaf volumes for three of the four remaining samples, Kharkof from Hays, Kansas, in 1943, Blackhull from Amarillo, Texas, in 1942, and Chiefkan from Lincoln, Nebraska, in 1940, are subnormal; whereas their mixing times only approach critical values.

It is pertinent to point out that during the drought years in the hard winter wheat area, particularly in 1935 and 1936, the mixing times for all varieties were greatly increased, thus indicating that the protein was wetted with much more difficulty when dehydrated to an excessive degree. Thus the somewhat above-critical mixing times for Kharkof from Hays and Blackhull from Amarillo (Table III) may be the result of drought conditions such as are known to have prevailed during the growing of the 1943 crop at Hays. The temperatures during the fruit-

ing period (studies to be reported in a later publication) were sufficiently high to account for the subnormal loaf volumes of these two samples.

These data suggest that the factor causing subnormal mixing times also will result in poorer protein quality as reflected in lower loaf volumes, provided it operates to the extent of producing sufficiently subnormal mixing times. The property of mixing time is regarded as being associated with or related to the physical and/or chemical constitution of the protein of any given variety. It appears, therefore, that there is a critical mixing time, characteristic of each variety, below which the physical constitution of the protein is definitely different from that characterized by the longer but still subnormal mixing requirements. In addition, the loss of normal elasticity and gas-retaining properties associated with subnormal loaf volumes indicates that the protein structure has been weakened. The fact that variety flours with subnormal loaf volumes almost invariably have critically subnormal mixing times appears to justify the use of mixing time as a means of deciding which samples are satisfactory for quality testing in the same way that test weight is now used.

**Acknowledgment**

The assistance of Dr. H. C. Fryer of the Statistical Laboratory of the Agricultural Experiment Station, Kansas State College, is gratefully acknowledged.

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## COMPARISON OF CORN STARCHES AT VARIOUS STAGES OF KERNEL MATURITY[1]

M. J. WOLF, M. M. MACMASTERS, J. E. HUBBARD, and C. E. RIST[2]

### ABSTRACT

The starch content in the grain of Iowa Hybrid 939, Iowax 1, and Golden Cross Bantam varieties of corn increased in the interval from 12 or 13 days after pollination to maturity. The most rapid increase occurred in the period from 12 to 20 days after pollination. The average of the starch granule diameter in the period from 13 days after pollination to grain maturity rose from 2.7 to 9.9$\mu$ in the nonwaxy dent corn, and from 3.0 to 9.1$\mu$ in the waxy corn. In the sweet corn, the corresponding values for the simple granules were 1.8 to 3.6$\mu$. The water-binding capacity of the starches at 12 to 13 days was approximately 0.9 g. per g. of starch and diminished rapidly to about 0.3 g. per g. at 20 to 36 days. Over the period studied, the iodine-sorptive capacity of the nonwaxy corn starches increased from 10 to 14 mg. per g. to 54 mg. per g. A low amylose content in starch from immature corn was verified by fractionation of starch from sweet corn 12 days after pollination. Two series of curves were obtained for Iowa Hybrid 939 and Iowax 1 corn starches, respectively, by plotting light transmittancy against temperature as dispersions of the starches were heated. Simple sigmoid curves were obtained for all samples of both series of starches except the mature, nonwaxy, dent corn starch. The latter gave a transmittancy curve with two inflections.

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Reports on the rate of formation, granule size, amylose to amylopectin ratio, and other characteristics of starch during the maturation of the corn kernel are quite limited. Evans (9) prepared photomicrographs of granules of yellow field corn starch at four stages of development to show the increase in diameter and the change from circular to polygonal outline of the granules with increase in kernel maturity. Hixon *et al.* (14) found that the viscosity of starch from corn at early stages of maturity was low and that it increased sharply at 4 to 5 weeks after pollination of the corn.

During the past several years considerable quantities of immature corn have reached the industrial market. Weather conditions unfavorable for maturing the crop and the practice of planting corn which requires a long growing period were major factors leading to this result. The effect of granule size upon processing yield and the suitability for industrial use of starch from immature corn have, consequently, made further knowledge of such starch a matter of practical significance.

Investigations were undertaken, therefore, to determine the approximate rate of starch formation in corn endosperm and some comparative physical and chemical properties of the starch granules at several stages of maturity of the corn.

## Materials and Methods

Iowa Hybrid 939 dent corn, Iowax 1 hybrid waxy dent corn, and a sweet corn variety, Golden Cross Bantam, were selected for study. Plantings were made in plots at the laboratory during three growing seasons, but work was confined chiefly to samples grown in 1945 and 1946.

The corn was pollinated by hand and samplings were made at various times from 12 to 13 days after pollination to maturity. Since about 24 hours are required for the pollen tube to reach the embryo sac, fertilization occurred about 1 day after pollination.

The ears of corn were collected between 8:00 and 9:00 a.m. and shelled immediately. Kernels of 12- and 13-day corn were frozen on solid carbon dioxide after shelling. At all other immature stages, the entire ears were frozen on solid carbon dioxide before shelling. All immature samples were kept frozen until they were processed for starch.

Moisture in the kernels was determined by drying a sample overnight in a vacuum oven at 60° to 70°C., grinding to a fine powder, and drying aliquots in a vacuum oven for 6 hours at 100°C. Moisture in the starch was determined by drying *in vacuo* in an Abderhalden drier for 4 hours at 100°C.

Nitrogen was determined by a modification of the Kjeldahl-Gunning-Arnold method (1) in which the ammonia was distilled into a boric acid solution (20).

Starch was determined polarimetrically in a calcium chloride extract of the dry, ground corn (8). Starch was separated from the samples of the 1945 crop year by the second method described by MacMasters and Hilbert (15). The starch obtained by this method was low in total nitrogen, but part of the small-granule fraction was lost in discarding the gluten layer.

In 1946 and 1947 a method was used by which an essentially quantitative separation was made of the starch from the kernel. The early samples, through 35 days after pollination, required no steeping. Mature samples of Iowa Hybrid 939 variety corn were steeped in distilled water for 3 days at 49°C. The starch granules showed no microscopic evidence of enzymic attack. Corresponding samples of waxy and sweet corn were steeped for 45 to 48 hours in a solution having an initial sulfur dioxide concentration of 0.2%.

Disintegration of the kernels was effected in iced distilled water (7 to 8 ml. per g. of corn) in a high-speed blendor. The fine material was separated by passing it through No. 17 standard silk bolting cloth. The residual wet pulp of the mature samples, which still contained much starch, was passed through a hammer mill. The pulp of Iowa Hybrid 939 variety and sweet corn was first steeped overnight in potassium hydroxide solution at pH 10.5 to 11.5. The ground material was washed on No. 17 standard silk bolting cloth.

The combined washings were centrifuged and when a lower layer of pure starch had formed it was separated from the upper layer of small starch granules, protein, and fiber. This upper layer, or in some cases the entire sample, was treated two or three times alternately with 80% ethanol and potassium hydroxide solution at pH 10.5 to 11.5 to remove protein. Fiber was separated from the starch by gravity sedimentation and by washing on No. 17 standard silk bolting cloth. The starch so obtained was combined with any starch originally obtained by centrifuging. Microscopic examination of the fiber fractions showed negligible loss of starch. After correcting for protein and moisture, the weight of starch recovered by this method was taken as a measure of the starch content of the grain. Protein was calculated by multiplying the total nitrogen by the factor 6.25.

Microscopic measurements of granule size were made only on starch from corn grown in 1946. Each sample for measurement of granule size was drawn with a large-orifice pipette from a suspension in which the starch granules were kept uniformly distributed by

rapid stirring. A drop of the suspension was mixed thoroughly with a drop of glycerol on a microscope slide and covered with a cover slip.

A microscope equipped with an 0.85 N.A. apochromatic objective and a 15X compensating eye piece was used for measuring granule size. Camera lucida drawings were made on graph paper of all granules in 3 to 10 fields, usually more than 5, selected at random from as many slides. Each granule was brought into focus before it was drawn. The magnification of the drawings was determined with the aid of a stage micrometer.

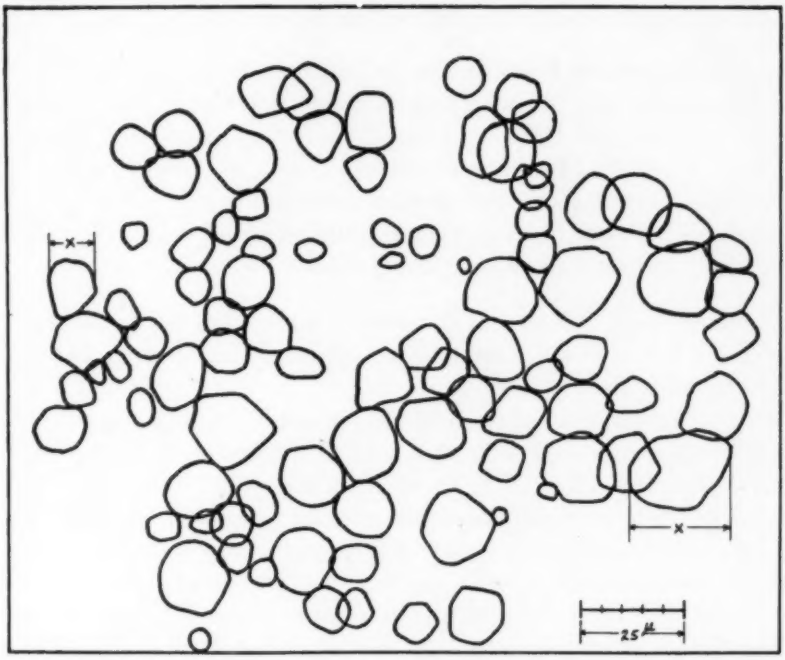


Fig. 1. Camera lucida drawing of a selected field of starch granules from mature Iowa Hybrid 939 dent corn. Granules were measured by their projection on the horizontal axis. × 1150.

Statistical diameters (6) were determined by measurement of the drawings. Because of the great spread in granule size, samples of from 530 to 2,483 granules were used. A selected field illustrating the method of granule diameter measurements is given in Fig. 1. The size frequency distribution of the starch granules shown here roughly approximates that of the mature starch of Iowa Hybrid 939 dent corn. The frequency distribution curve derived from a sample of 959 starch granules of this variety is shown in Fig. 3.

The water bound by the freshly prepared, undried starch before treatment with ethanol and potassium hydroxide was determined by the refractometric method of Dumanski (7). The method is based on

the hypothesis that bound water does not act as a solvent for dissolved solutes. The starch sample was mixed with a sucrose solution of known concentration. After the water in the system had attained an equilibrium distribution with respect to the water bound by the starch and that acting as solvent for sucrose, the change in sucrose concentration was determined refractometrically. The water bound by starch in this equilibrium mixture was calculated by Dumanski's equation for samples free of solutes,

$$X = \frac{ap}{100} + B\frac{b_2 - b_1}{b_2},$$

where $X$ = grams of bound water in $p$ grams of original sample,
$a$ = per cent of water in sample (determined by drying *in vacuo* at 100°C),
$p$ = weight of sample in grams,
$B$ = grams of original sucrose solution,
$b_1$ = per cent of sucrose in original solution,
$b_2$ = per cent of sucrose in equilibrium solution.

Any effect on the bound-water content of the samples due to protein contamination was not taken into account in calculating the water bound by the starch.

Only starch separated from kernels of the 1946 crop by the quantitative method was used. From 0.1 to 2.1 g. (dry basis) of starch were shaken with 4 to 12 g. of 10 to 60% sucrose solution for 30 minutes. The suspension was centrifuged to remove the starch prior to determination of the refractive index of the equilibrium solution. In 60% sucrose solution, the specific gravity was sufficiently high that the starch did not sediment and filtration was required. In general, the values found for the bound-water content of starch decreased as the sucrose concentration of the equilibrium solution was increased.

Starch was defatted prior to fractionation and to determination of iodine-sorptive capacity by extraction for 48 hours in a Soxhlet- or Butt-type extractor with 85% methanol in the extraction flask.

As a measurement of the amylose content, the iodine-sorptive capacity of the defatted starches was determined by the method of Bates, French, and Rundle (2), as modified by Wilson, Schoch, and Hudson (19). Determinations were made on immature Iowa Hybrid 939 and sweet corn starches both before and after the separation of most of the contaminating protein. There was no interference with the determination by as much as 20% protein in the sample.

Starch was fractionated by a modification (cf. 13, J. E. Hodge, unpublished data) of Schoch's butanol precipitation method (17), but

the crude amylose was autoclaved only 10 to 15 minutes in the purification procedure. The purification process was repeated until the iodine-sorptive capacity of two successive precipitates agreed within 1%. The amylopectin solution was concentrated under reduced pressure, below 50°C., and the amylopectin was precipitated from the aqueous concentrate by adding it slowly to absolute ethanol. It was then dried in a vacuum desiccator over calcium chloride, after which drying was completed in a vacuum oven at 70°C.

The light transmittancy of starches during pasting in water was followed by the method of Morgan (16) with apparatus modified by S. A. Karjala (unpublished). Light transmittancy data were obtained only for the starches prepared in the 1945 season.

## Results and Discussion

Moisture content of the corn kernels decreased from about 87% at 12 to 13 days after pollination to 9 to 11% at maturity (Table I).

TABLE I

AGE AND MOISTURE CONTENT OF GRAIN

| Variety | 1946 Age,[1] days | 1946 Moisture content, % |
|---|---|---|
| Iowa Hybrid 939 dent corn | 13 | 86.8 |
| | 15 | 86.2 |
| | 20 | 78.9 |
| | 35 | 55.6 |
| | Mature[2] | 11.2 |
| Iowax 1 hybrid waxy dent corn | 13 | 86.4 |
| | 15 | 84.5 |
| | 20 | 78.4 |
| | 35 | 61.3 |
| | Mature[2] | 10.9 |
| Golden Cross Bantam variety sweet corn | 12 | 86.5 |
| | 14 | 80.5 |
| | 20 | 76.3 |
| | 35 | 60.9 |
| | Mature[2] | 9.0 |

[1] Age is defined as the number of days after pollination.
[2] Mature corn samples were harvested 70 to 74 days after pollination.

Over the same period, the amount of starch in the corn increased (Fig. 2). The rise in starch content in all three varieties was most rapid between 12 and 20 days after pollination. All of the varieties of corn studied contained less starch than was found by Evans (10) in his study of the maturation of Minnesota Hybrid 403 dent corn.

The polarimetric method of analysis indicated higher starch content at most stages of maturity than was obtained by separation (Fig. 2). Part of this difference resulted from a slight loss of starch during

recovery, but most of it is attributable to the fact that the polarimetric procedure measures both starch and soluble polysaccharides. This was especially evident in sweet corn in which less than 50% of the total polysaccharides was recovered as starch. The yield of 26% starch obtained from mature Golden Cross Bantam sweet corn was in good agreement with the 21 to 25% starch content of Golden Bantam sweet corn reported by Sumner and Somers (18).

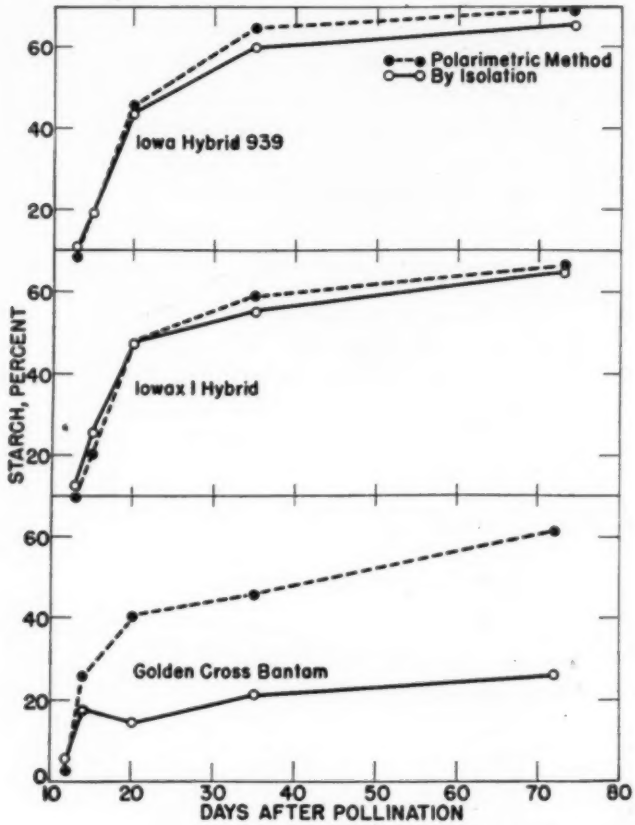


Fig. 2. Starch content of corn at different stages of kernel maturity (dry basis).

The total nitrogen content of the starches prepared by the quantitative procedure was high in comparison with that of starches prepared by the method in which part of the small granules were lost into the tailings fraction (Table II).

Two types of series of granule size distribution curves were obtained (Fig. 3). Beginning with a narrow range of granule diameters at 13 days after pollination, the granule size frequency distribution curves of the two dent corn starch varieties showed a marked spread and a

rapid shift of the peak as the corn increased in maturity. The sweet corn starch curves, on the other hand, showed little change in spread or in the position of the peak. This difference is attributable to the fact that in the varieties studied the dent corns contain simple starch granules which vary considerably in size, while the sweet corn starch

TABLE II

IODINE-SORPTIVE CAPACITY AND TOTAL NITROGEN CONTENT OF STARCH

| Variety | Time of sepn. of starch, days after pollination | Iodine-sorptive capacity, mg. per g. starch (d.b.) | | Total N, % (d.b.) | |
|---|---|---|---|---|---|
| | | 1945 | 1946 | 1945 | 1946 |
| Iowa Hybrid 939 dent | 13 | — | 13 | — | 1.4 |
| | 15 | 29 | 22 | 0.04 | 1.2;[1] 0.28 |
| | 18 | 40 | — | 0.03 | — |
| | 20 | — | 41 | — | 1.1;[1] 0.24 |
| | 21 | 42 | — | 0.03 | — |
| | 35 | — | 50 | — | 0.91;[1]0.20 |
| | Mature | 48 | 53 | 0.06 | 0.42 |
| Iowax 1 hybrid dent | 13 | — | 5.4 | — | 1.13 |
| | 15 | 0.8 | 2.9 | 0.01 | 0.97 |
| | 18 | 0.8 | — | 0.02 | — |
| | 21 | 0.7 | — | 0.02 | — |
| | Mature | 0.9 | 2.5 | 0.04 | 0.29 |
| | | 1946 | 1947 | 1946 | 1947 |
| Golden Cross Bantam sweet | 12 | 10 | 24 | — | 0.15 |
| | 14 | 28 | — | 1.7;[1]0.85 | — |
| | 20 | 32 | — | 3.5;[1]1.9 | — |
| | 35 | 54 | — | 3.2;[1]1.1 | — |
| | Mature | 53 | — | 0.29 | — |

[1] Values before and after treatment, respectively, with 80% ethanol and KOH solution at pH 10.5 to 11.5. These starches were prepared by the quantitative method.

granules are chiefly compound. These compound granules break during processing into simple granules which are relatively uniform in size. Results on the sweet corn starch at 20 days after pollination are out of line, probably because this sample came from corn that was pollinated and harvested out of sequence with the other corn samples.

The average granule diameter of the dent corn starches increased most rapidly over the period 13 to 20 days after pollination, after which the increase was slower (Fig. 4). For sweet corn starch, the rate of increase of average granule diameter was less than for the dent corn starches, and was approximately linear over the entire period studied.

Starch granules from mature Iowa Hybrid 939, Iowax 1, and Golden Cross Bantam varieties were found to have average diameters of 9.9, 9.1, and 3.6$\mu$, respectively.

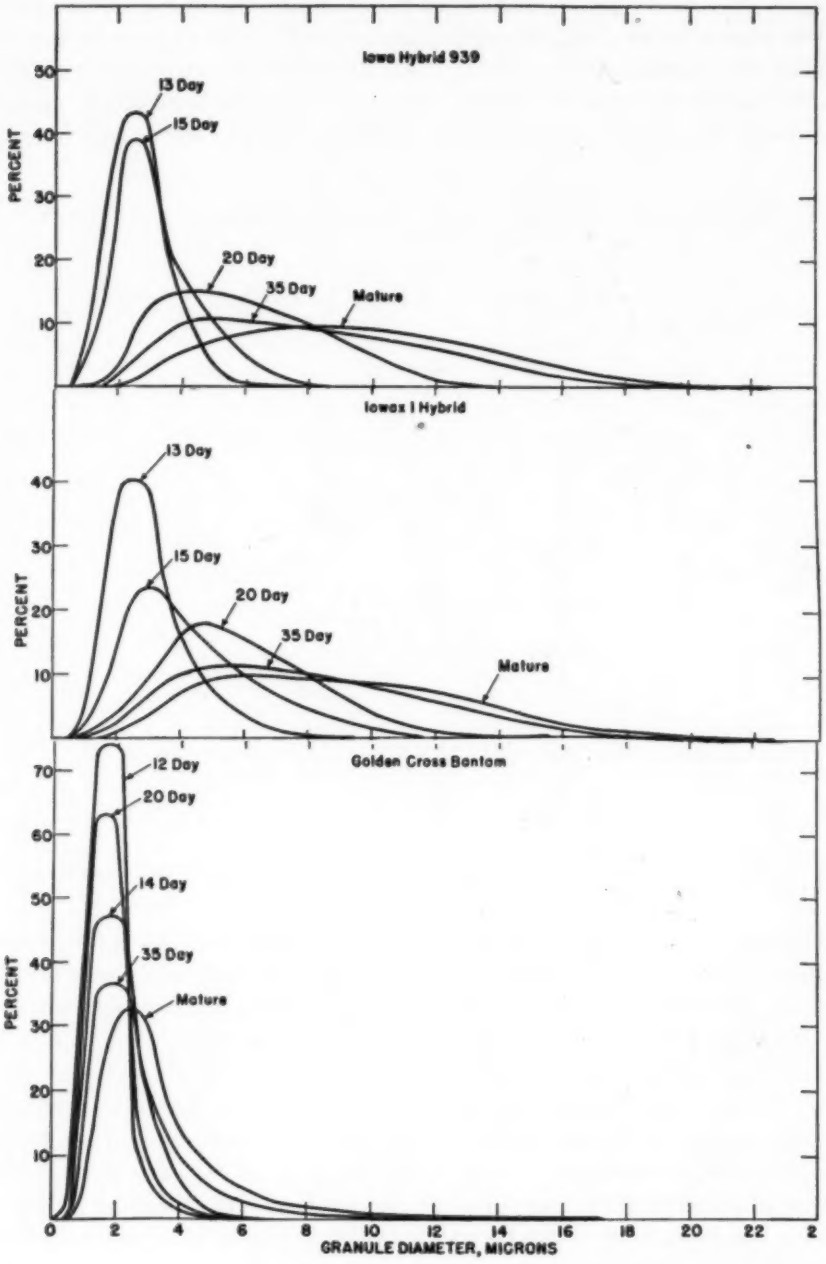


Fig. 3. Size frequency distribution curves of starch granules at different stages of kernel maturity. The curves were smoothed from histograms with an interval of 1μ.

The water-binding capacity of the three varieties of corn starch granules in equilibrium with 10 to 60% sucrose solution diminished rapidly over the period 12 to 20 days after pollination. The water-binding capacity during this period decreased to a greater extent than

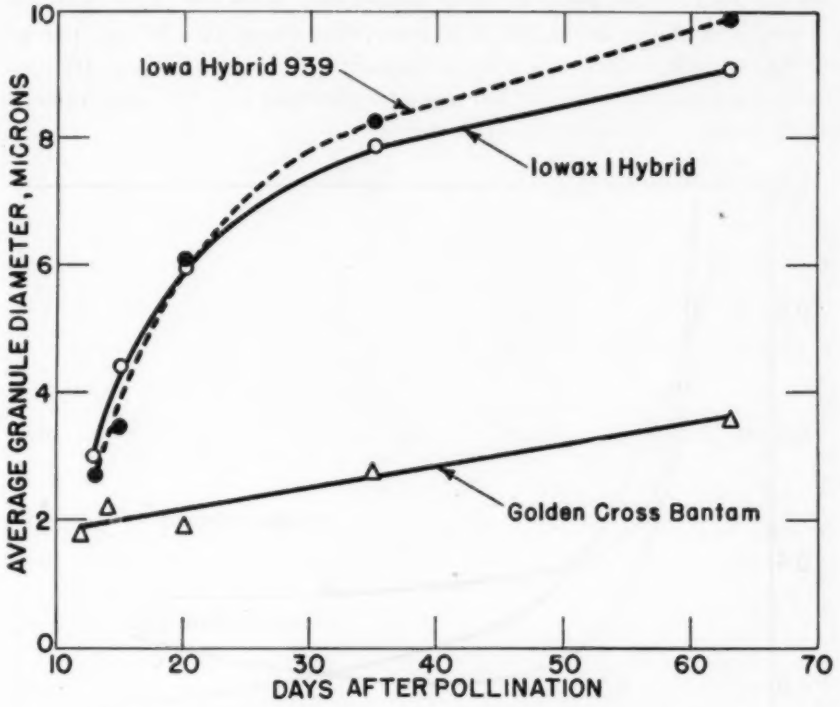


Fig. 4. Average starch granule diameters at different stages of kernel maturity.

did the mosture content of the kernel (Table I). Typical data (Fig. 5) showed that in equilibrium with 10% sucrose solution the starch bound about 0.9 g. of water per g. at 12 to 13 days after pollination. At 20 days, the corresponding value was approximately 0.3 g. per g. Chesheva (5) reported approximately 0.12 g. of water bound per g. of corn starch, from mature corn, in equilibrium with 10% glucose solution. Potato starch was found by Freeman (11) to bind an average of 0.31 g. of water per g. in equilibrium with sucrose solutions of different concentrations.

Starch from immature corn of the nonwaxy varieties had a lower iodine-sorptive capacity, indicating a lower ratio of amylose to amylopectin, than the corresponding starch from mature corn. Waxy corn starch had the anticipated low iodine-sorptive capacity, characteristic of the absence of amylose, throughout the maturation period of the corn (Table II). From 12 to 13 days after pollination to maturity, the

iodine-sorptive capacity of sweet corn starch increased by approximately a factor of five, while that of nonwaxy dent corn starch increased about fourfold.

Fractionation of starch from sweet corn 12 days after pollination yielded 10.5% amylose, in good agreement with the 12% amylose content calculated from the iodine-sorptive capacity (24 mg. per g.) of the sample. Iodine-sorptive capacity of the amylose fraction reached a limiting value of 200 mg. of iodine per g. The amylopectin fraction sorbed 9.3 mg. of iodine per g.

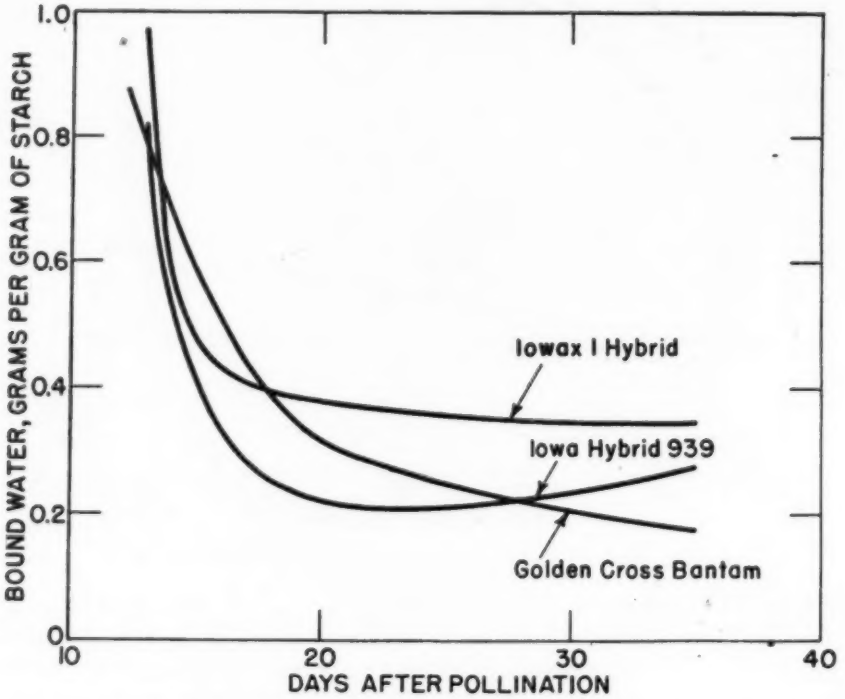


Fig. 5. Bound water content, on a dry weight basis, of corn starches at different stages of kernel maturity, in equilibrium with 10% sucrose solution.

There were appreciable differences in iodine-sorptive capacity of comparable starches separated from corn grown in different years (Table II). These differences cannot be explained on the basis of present knowledge.

Current information concerning the enzymic synthesis of starch is not sufficient to afford a clear explanation for a progressive increase in the proportion of linear molecules in starch as the grain matures. Bourne and Peat (4) considered at least two enzymes, P and Q, to be involved in the conversion of glucose-1-phosphate to a product similar

to amylopectin. Phosphorylase (the P enzyme) and glucose-1-phosphate yield a linear glucose polymer. The Q enzyme is believed to catalyze the formation of amylopectin from amylose through the hypothetical intermediate, "pseudoamylose." Bourne and Peat expressed the opinion that the ratio of the P and Q enzymes determines whether amylose, amylopectin, or a mixture of the two will be produced.

If a linear polymer is the precursor of both amylose and amylopectin, the enzyme system must favor further polymerization to a nonlinear molecule more in the immature grain than it does in grain nearing maturity. Possibly there is circumstantial evidence for this view in the relatively large amount of water-soluble polysaccharide, apparently similar to glycogen (12), present in immature sweet corn. Starch granules lie embedded within globules of this material, which suggests that this water-soluble polysaccharide may be an intermediate in starch formation.

Morgan (16) noted that suspensions of starch with large granules transmit more light during the pregelatinization period than suspensions with small granules. It was recently reported by Beckord and Sandstedt (3) that the light transmittancy of the large-granule fraction of wheat starch is greater than that of the small-granule fraction over the entire heating range. The granule size frequency distribution curves of Fig. 3 do not apply to the corn starches which were prepared in 1945, because the small granules were not adequately represented. Despite this fact, there is sufficient variation in granule size at the different stages of maturity to show clearly the dependence of light transmittancy on the granule size. Waxy corn starches gave simple sigmoid curves (Fig. 6) which showed an increase in light transmittancy over the entire temperature range with increase in maturity of the grain. Nonwaxy dent corn starches showed a correlation between light transmittancy and granule size only in the pregelatinization range. Only the starches from immature kernels of this series gave curves which were sigmoid in character. At maturity the curve showed two inflections with a region of relatively low light transmission between 75° and 90°C.

The most rapid changes in starch properties were found to occur during the first 35 days after pollination. Changes in water-binding capacity and iodine-sorptive capacity which occurred after this period were probably too small to be of significant effect upon industrial processing of corn and utilization of the starch. From the standpoint of commercial starch production, however, changes in starch content of the grain and in granule size of the starch might be of significance to the processing industry. In Iowa Hybrid 939 corn, for example, the

starch content was approximately 10% less at 35 days after pollination than at maturity. At the earlier date about 66% of the granules were less than 10 $\mu$ in diameter, while in mature corn 53% of the granules were in this range

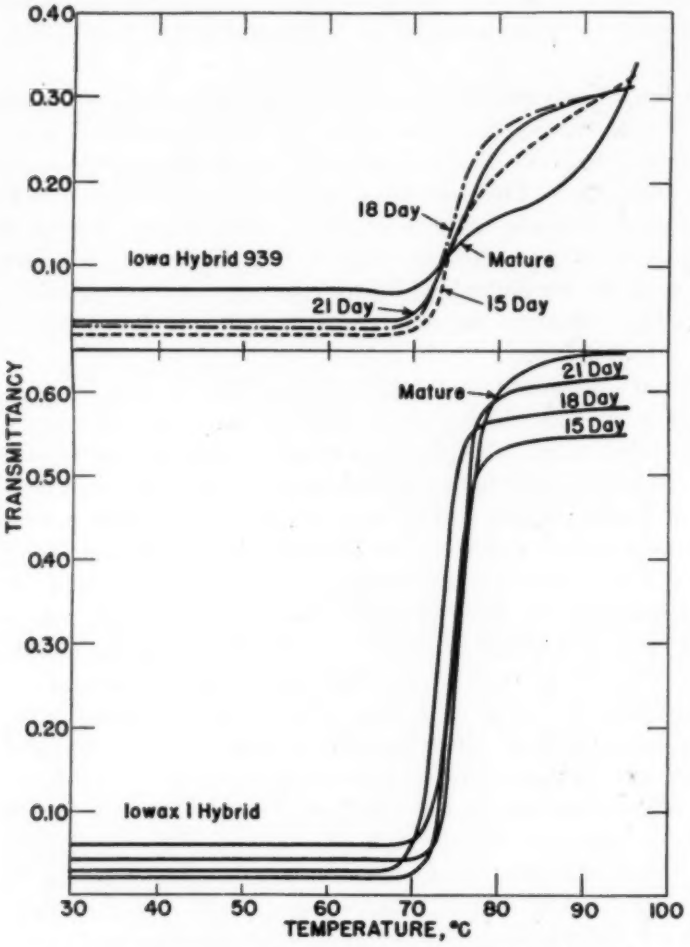


Fig. 6. Light transmittancy curves of 0.1% suspensions of corn starches at different stages of kernel maturity.

Although small granules are in the majority on a number basis, calculations on a weight basis, assuming an average density of 1.63 for corn starch, show the minor contribution of the small granules to the total weight of the starch. The granules having diameters up to 10 $\mu$ account for 21% of the weight of the starch at 35 days after pollination and 15% of the weight of the starch at maturity. Thus it is apparent that since the smaller granules are the most difficult to

recover in processing, the loss of starch in commercial processing would be greater with immature corn.

**Acknowledgment**

We are indebted to F. R. Earle and others of the Analytical and Physical Chemical Division of this laboratory for the analyses for moisture and starch in corn. The assistance of Carol M. Jaeger in the statistical analyses and of Margaret N. Mason, John A. Cannon, Beatrice Jacobs, Margaret Holzapfel, John Warner, and General Irving in the laboratory work is gratefully acknowledged.

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# INFLUENCE OF PROTEOLYTIC ENZYMES AND YEAST NUTRIENTS UPON THE REQUIREMENT FOR MALT IN GRAIN ALCOHOL FERMENTATIONS [1]

J. M. VAN LANEN [2], E. H. LEMENSE [3], ABE ANELLIS [4], and JULIAN CORMAN

**ABSTRACT**

The value of proteolytic enzymes, protein hydrolyzates, vitamins and other growth factors for yeast as partial replacements for barley malt in grain alcohol fermentation was determined. Slurries of corn or wheat were premalted at 70°C. for 30 minutes with malt equivalent to 1% of the grain bill, after which mashes were cooked at 25-lbs. pressure for 30 minutes. The cooked mashes were cooled and varying quantities of conversion malt and adjuncts were added. After holding at 58°–60°C. for 30 minutes the temperature was lowered to 30°C., and 2% by volume of distillers' yeast NRRL No. Y-567 was added. The action of adjuncts upon both the rate of fermentation and the final yield of alcohol was followed.

Papain, ficin, culture filtrates from *Bacillus subtilis*, and protein hydrolyzates were found capable of substituting for a portion of the conversion malt. When these were supplied at their optimum concentration equivalent yields of alcohol and rapid fermentation rates were obtained with 60% of the normal amount of malt. Some beneficial effect was noted by adding the same materials to yeast seed cultures. It is shown that the effect of proteolytic enzymes is associated with the nutrition of the yeast rather than with the release or activation of malt amylases.

To meet urgent wartime demands, industrial alcohol was manufactured from 1943 through 1945 at a rate about fivefold over prewar production. While fermentation of molasses and synthesis from ethylene provided a portion of our wartime alcohol requirement, the bulk of it was supplied by processing grain and grain products (1). This increase in the utilization of grain required correspondingly greater quantities of barley malt, and for a time curtailment of grain alcohol production was imminent owing to a shortage of distillers' malt.

In view of this contingency, an investigation was undertaken to determine whether the amount of barely malt, which normally comprises 8 to 10% of the grain entering the distillery, could be reduced without sacrificing the yield of alcohol or unduly prolonging the fermentation time. The present study is concerned with the effects of proteolytic enzymes and yeast nutrients upon the malt requirement in corn and wheat mashes used for alcohol production.

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The liberation or activation of bound amylases by proteolytic enzymes has long been known, and this property is commonly utilized in determining the total amylase activity of cereals (Myrback and Myrback (5), Snider (8)). Likewise, numerous investigators have recognized the stimulatory properties of protein degradation products upon yeast growth. These studies recently reviewed (Thorne (10)) have revealed that available ammonium salts, asparagin, aspartic acid, glutamic acid, leucine, and arginine are outstanding in their stimulatory action. The remaining amino acids vary appreciably in the response which they elicit with a few actually being inhibitory. With regard to peptides, availability to the yeast cell decreases as the length of peptide chain increases with the result that proteins and high molecular weight peptides are virtually without effect. The nitrogen nutrition of yeasts has been examined largely through the use of synthetic and grain wort media, and the knowledge gained therefrom has not been applied extensively to alcoholic fermentation of whole grain mashes.

## Methods

For most of these experiments, grain mashes were prepared by weighing 45.0 grams of ground corn or wheat and 0.5 gram of barley malt into 500 ml. flasks and adding 170 ml. of water at 70°C. to each flask. The flasks were placed in a water bath at 70°C. and the grain slurries were stirred intermittently during 30 minutes. Following this premalting, mashes were cooked in the autoclave for 30 minutes at a steam pressure of 25 pounds. After cooking, mashes were cooled to 70°C. and the malt, slurried with 50 ml. of water, was added. The flasks were then placed in a water bath adjusted to 58–60° and conversion was continued for 30 minutes during which the mashes were agitated frequently. With mashes saccharified with malt plus adjunct where less conversion malt was used, a correspondingly larger quantity of corn or wheat was mashed. Converted mashes were cooled to 30°C. and inoculated with 2% by volume of a 24-hour culture of distillers' yeast NRRL strain No. Y-567. The final volume in each flask was approximately 250 ml.

Fermentation was conducted at 30°C. Progress was followed either by weighing the flasks or determining their alcohol content. At 72 hours, unless otherwise noted, the beers were brought to a volume of 300 ml. and a 200-ml. portion was taken for determination of alcohol. One hundred ml. of distillate was collected from the 200 ml. of beer and the alcohol concentration in the distillate was measured by reading the refractive index with a dipping refractometer. Data reported are in all cases the averages of duplicate flasks.

Alcohol yields are expressed as proof gallons per 56 pounds of total grain as used. The wheat employed had the following percentage composition: Moisture, 11.6; starch (A.O.A.C.), 59.4; sugars as glucose, 1.9; pentosans, 5.6; and nitrogen, 2.06. The percentage composition of the corn was: Moisture, 10.5; starch (polarimetric), 63.6; sugar as glucose, 1.77; and nitrogen, 1.49. The barley malt was a commercial sample with 180° Lintner value. Its percentage composition was: Moisture, 8.05; starch (polarimetric), 40.7; sugar as glucose, 9.11; and nitrogen, 2.22.

## Experimental

*Papain in Wheat Mashes.* Wheat mashes prepared as described, except for the use of larger volumes to permit frequent sampling, were converted with (a) 8% malt, (b) 5% malt, (c) 5% malt plus varying levels of papain, and (d) 5% malt plus similar levels of heat-inactivated papain. After conversion and cooling, the mashes were inoculated and allowed to ferment 50 hours. Fermentation progress was followed by periodically determining the alcohol content of the mashes.

TABLE I

THE EFFECT OF ADDING PAPAIN AND HEAT-INACTIVATED PAPAIN TO WHEAT MASHES ON THE RATE OF FERMENTATION AND YIELD OF ALCOHOL

| Papain added | Malt added | Alcohol production | | | |
|---|---|---|---|---|---|
| | | Percent by volume after | | | Final yield |
| Mg./100 ml. | Percent of grain | 18 hours | 40 hours | 50 hours | Proof gal./bu. |
| Untreated papain | | | | | |
| None | 8 | 3.50 | 7.14 | 7.30 | 4.84 |
| None | 5 | 2.70 | 6.04 | 6.57 | 4.41 |
| 1 | 5 | 3.47 | 6.82 | 7.03 | 4.72 |
| 10 | 5 | 5.38 | 7.07 | 7.17 | 4.82 |
| 20 | 5 | 5.49 | 7.07 | 7.31 | 4.92 |
| 50 | 5 | 5.68 | 7.04 | 7.31 | 4.92 |
| 100 | 5 | 5.74 | 7.10 | 7.20 | 4.83 |
| Heat-inactivated papain [1] | | | | | |
| 1 | 5 | 2.74 | 6.04 | 6.70 | 4.51 |
| 10 | 5 | 2.66 | 6.08 | 6.67 | 4.47 |
| 20 | 5 | 2.66 | 6.15 | 6.68 | 4.48 |
| 50 | 5 | 2.74 | 6.00 | 6.56 | 4.41 |
| 100 | 5 | 3.03 | 6.36 | 6.90 | 4.63 |

[1] Papain was inactivated by autoclaving at 121°C. for 15 minutes.

Results presented in Table I show clearly tnat in mashes receiving papain, fermentation started much earlier than in the controls which were converted with either 5 or 8% malt. After 18 hours the alcohol concentration in papain mashes was approximately double that in the

5% malt control. While the difference became less pronounced as fermentation continued, nevertheless, upon final analysis mashes converted with 5% malt plus either 20 or 50 mg. % papain gave yields equivalent or slightly higher than the 8% malt control. Those receiving 10 and 100 mg. % papain were nearly equivalent to the 8% malt control while the addition of as little as 1 mg. % papain resulted in a significant improvement over the 5% malt control. With heat-inactivated papain there was little or no stimulation of fermentation, except for a small increase with the highest level, due possibly to the presence of heat-stable nutrients contained in the enzyme preparation.

*Stimulation by Other Proteolytic Enzymes.* The action of ficin and the proteinase from *Bacillus subtilis* (scaber strain NRRL B-544) on the fermentation of grain mashes is shown in Table II. Proteinase production by this organism cultured on the surface of asparagus butt medium has been described by Kline *et al.* (4). In preliminary experiments it was found that very active proteolytic preparations could be produced by growing the organism under submerged aerobic culture conditions in a medium composed of thin grain stillage, glucose, and calcium carbonate. The proteolytic culture liquor used in this experiment was obtained by cultivation of *B. subtilis* NRRL B-544 for 72 hours in thin grain stillage supplemented with 2% glucose and 1% calcium carbonate. This culture liquor was filtered but no attempt was made to effect complete removal of the bacteria.

Duplicate flasks of corn mash, after cooking and cooling, were converted with the following preparations: 10% malt; 4% malt; 4% malt plus papain; 4% malt plus ficin; 4% malt plus two levels of *B. subtilis* culture filtrate; and 4% malt plus untreated thin grain stillage.

Weight losses (Table II) show an increased rate of fermentation in mashes which received the culture liquor, papain, and ficin; the differences again were especially apparent after 18 and 27 hours. With continued incubation, the control mashes approached the enzyme-treated mashes with respect to their total loss in weight. Final alcohol yields after 66 hours of incubation showed that the papain and ficin-treated mashes, although considerably improved in fermentation efficiency over 4% malt mashes, were slightly lower than those in which 10% of malt was used. Mashes supplied with *B. subtilis* culture filtrate, however, were at least equivalent to those employing the high level of malt. Thin stillage slightly increased the rate of fermentation but did not significantly raise the final alcohol concentration. All mashes receiving adjuncts were less acid after fermentation. This condition invariably prevailed in proteolyzed mashes. It seemingly is caused either by the increased buffer action of the protein degradation products or by the more rapid utilization of sugar by the yeast, thus sup-

pressing growth and acid production by bacteria introduced with the malt.

Repeated trials wherein suboptimal levels of malt were supplemented with proteinases resulted in all cases in a shortened lag period and an increased rate of fermentation. The yield of alcohol was either equivalent or superior to that produced by 8% malt. These results indicated that proteinases were either liberating the malt amylases more completely, or they were supplying other substances which enhanced fermentation or saccharification, or both. A study was made, therefore, to determine whether the beneficial action of papain was attributable to its ability to release amylases or to provide yeast nutrients or whether both of these factors were involved.

TABLE II

THE EFFECT OF BACTERIAL AND PLANT PROTEINASES ON THE RATE OF FERMENTATION AND THE YIELD OF ALCOHOL FROM CORN MASHES CONVERTED WITH A SUBOPTIMAL LEVEL OF MALT

| Malt added percent | Adjunct | Weight loss in grams | | | | Final pH | Yield of alcohol Proof gal. /bu. |
|---|---|---|---|---|---|---|---|
| | | 18 hours | 27 hours | 50 hours | 66 hours | | |
| 10 | None | 7.2 | 10.7 | 16.4 | 17.2 | 4.2 | 5.05 |
| 4 | None | 5.2 | 7.6 | 14.5 | 16.0 | 4.1 | 4.70 |
| 4 | Thin stillage (8% of mash volume) | 5.8 | 8.4 | 15.0 | 16.2 | 4.3 | 4.74 |
| 4 | Papain (10 mg. %) | 11.4 | 13.5 | 15.5 | 16.5 | 4.5 | 4.91 |
| 4 | Ficin (5 mg. %) | 12.7 | 13.8 | 15.5 | 16.4 | 4.6 | 4.97 |
| 4 | *Bacillus subtilis* (4% of mash volume) | 13.4 | 14.4 | 16.2 | 17.2 | 4.8 | 5.03 |
| 4 | *Bacillus subtilis* (8% of mash volume) | 13.5 | 14.6 | 16.2 | 17.1 | 4.7 | 5.14 |

*Effect of Papain upon Saccharification.* Cooked corn mashes in double the quantity described under Methods were converted with: (a) 8% malt, (b) 5% malt, and (c) 5% malt plus 10 mg. % papain. Determinations of reducing sugars calculated as maltose were made before inoculation and at intervals during fermentation. Fermentation progress was also followed in separate pairs of flasks by determination of weight losses. Final alcohol concentration was measured in these flasks after a period of 50 hours. Results are plotted in Fig. 1.

By comparison of the sugar values after conversion, it is apparent that papain did not increase the primary saccharification rate. The sugar concentrations were nearly the same in mashes converted with 5% malt with and without papain, and slightly higher as might be expected with 8% malt. However, from the residual sugar concentrations during fermentation, it can be seen that in papain mashes, active fermentation began much earlier and continued at a more rapid rate

with the result that at 24 hours the sugar was almost completely utilized. Mashes without papain showed a definite lag period and required about 40 hours with 8% malt and about 45 hours with 5% malt to reach the same residual sugar level. The beneficial action of papain, therefore, is clearly associated with fermentation rather than with saccharification. This observation was substantiated by determining the alpha-amylase content of mashes converted in the presence and absence of papain, by a modification of the method of Olson,

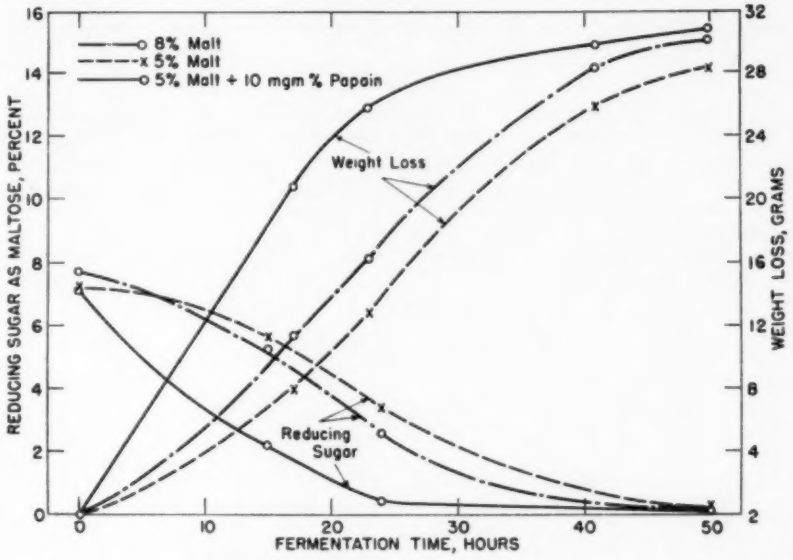


Fig. 1. Progress of alcoholic fermentation of grain mashes with and without papain.

Evans, and Dickson (6). As in the case of reducing sugar, no significant increase in alpha-amylase activity during the conversion period could be demonstrated by adding papain.

*Effect of Vitamins.* Papain is commonly used either singly or combined with other enzymes to liberate vitamins and growth factors prior to microbiological assay (3). To determine whether the yeast-stimulating action of papain could be explained by its ability to release yeast growth factors, the influence of adding various vitamins and vitamin concentrates was next studied. The following were added to corn mashes saccharified with 5% malt: Thiamin, riboflavin, niacin, pyridoxin, pantothenic acid, biotin, inositol, para-aminobenzoic acid, and a combination of these factors. Also supplied to separate mashes were dehydrated liver extract and dried distillers' solubles. Fermentation rates which were determined by weight losses and final alcohol yields revealed the single vitamins to be ineffective as stimulants and

the combined vitamins to be only slightly effective. Liver extract and dried solubles showed less stimulation than did papain.

*Effect of Papain on the Amino Nitrogen Content of Grain Mashes.* Two series of mashes were converted with 5% malt, 5% malt plus papain, and 10% malt. One series was inoculated with yeast in the normal manner while the other was incubated at the same temperature without addition of yeast. Malt slurries were pretreated with formalin to suppress bacterial activity. At intervals samples were withdrawn from each series of flasks for the determination of amino nitrogen content by the formol titration method. Results are shown in Table III.

TABLE III

THE INFLUENCE OF PAPAIN ON THE AMINO NITROGEN CONTENT OF CORN MASHES INCUBATED IN THE PRESENCE AND ABSENCE OF YEAST

| | Uninoculated mashes | | | Inoculated mashes | | |
|---|---|---|---|---|---|---|
| Malt concentration, % | 5 | 10 | 5 | 5 | 10 | 5 |
| Papain added, mg./100 ml. | None | None | 10 | None | None | 10 |
| Amino nitrogen content, mg./100 ml. | | | | | | |
| 0 hr. | 4.9 | 5.6 | 7.0 | 4.9 | 5.6 | 7.0 |
| 16 hr. | 5.3 | 5.9 | 7.2 | 1.4 | 2.1 | 2.5 |
| 48 hr. | 5.5 | 6.6 | 8.5 | 0.8 | 1.0 | 2.0 |
| 72 hr. | | | | 1.1 | 1.3 | 3.7 |
| 116 hr. | 6.2 | 7.7 | 9.8 | | | |

It may be seen from these data that there is an increase in the amino nitrogen content of uninoculated mashes during incubation due probably to the proteolytic activity of the barley malt. It is also apparent that papain, as might be anticipated, appreciably increased the amino nitrogen content of mashes saccharified with 5% malt. Such mashes contained even more amino nitrogen than those converted with 10% malt. Of more significance is the fact that most of this nitrogen is available to the yeast, as indicated by the sharp decrease in the amino nitrogen levels in inoculated mashes.

While the amino nitrogen content of papain-treated mashes was not reduced during fermentation to as low a level as in mashes containing only malt, the total utilization of nitrogen was greater in the former. The utilization of such a high proportion of amino nitrogen in all the mashes, and especially in those with suboptimal quantities of malt, suggested that lack of available nitrogen might limit to some extent the activity of the yeast. This possibility was investigated further by adding various quantities of amino nitrogen to mashes converted with insufficient malt.

*Effect of Adding Organic Nitrogen.* Corn mashes were saccharified with 10% malt, 4% malt, and 4% malt plus varying increments of

acid-hydrolyzed casein, urea, and thin grain stillage. After inoculation, the rate of fermentation and final yields of alcohol were recorded.

Results shown in Table IV demonstrate the complete replaceability of papain with casein hydrolyzate. Although urea produced a similar effect, its use did not result in equivalent alcohol yields and it sharply depressed alcohol formation when used in greater quantities. Casein hydrolyzate, when added to supply from 12 to 24 mg. of amino nitrogen per 100 ml. of mash, stimulated fermentation similar to papain added

TABLE IV

THE EFFECT OF CASEIN HYDROLYZATE, UREA, AND STILLAGE ON THE FERMENTATION OF CORN MASHES SACCHARIFIED WITH A SUBOPTIMAL LEVEL OF MALT

| Conversion malt | Nitrogen supplement per 100 ml. of mash | Weight loss in grams | | | Final pH | Yield of alcohol |
|---|---|---|---|---|---|---|
| | | 18 hours | 42 hours | 68 hours | | Proof gal./bu. |
| 10 | None | 7.5 | 15.5 | 16.7 | 4.3 | 5.17 |
| 4 | None | 5.6 | 13.6 | 15.9 | 4.1 | 4.76 |
| 4 | 20 ml. stillage | 7.5 | 13.8 | 16.2 | 4.2 | 4.92 |
| 4 | 10 mg. papain | 11.4 | 15.3 | 16.8 | 4.5 | 5.10 |
| | Casein hydrolyzate-Amino nitrogen equivalents | | | | | |
| 4 | 2 mg. | 7.1 | 14.4 | 16.2 | 4.3 | 5.04 |
| 4 | 6 mg. | 9.2 | 14.6 | 16.3 | 4.4 | 5.13 |
| 4 | 12 mg. | 10.7 | 14.5 | 16.4 | 4.5 | 5.14 |
| 4 | 24 mg. | 12.2 | 15.4 | 16.5 | 4.6 | 5.20 |
| 4 | 40 mg. | 12.7 | 14.7 | 16.1 | 4.6 | 5.05 |
| | Urea-Nitrogen equivalents | | | | | |
| 4 | 2 mg. | 6.7 | 12.8 | 15.8 | 4.3 | 4.83 |
| 4 | 6 mg. | 8.4 | 14.3 | 16.0 | 4.3 | 5.03 |
| 4 | 12 mg. | 9.3 | 14.2 | 16.0 | 4.4 | 4.89 |
| 4 | 24 mg. | 11.6 | 14.3 | 15.9 | 4.4 | 4.86 |
| 4 | 40 mg. | 12.3 | 14.1 | 15.7 | 4.5 | 4.68 |

at 10 mg. %. Furthermore, even when added at the rate of 2 and 6 mg. of amino nitrogen per 100 ml. of mash, casein hydrolyzate increased substantially both the yield of alcohol and the rate of fermentation. The rate of fermentation was raised still more, but the yield of alcohol was decreased, by the addition of the hydrolyzate to supply 40 mg. % amino nitrogen. Amino acids added singly to supply equivalent amounts of nitrogen were not as effective as casein hydrolyzates. With urea, the fermentation rate was increased slightly less than with casein hydrolyzate and the yield of alcohol was correspondingly lower. When added at a rate of 24 or more mg. of nitrogen per 100 ml., urea lowered the yield of alcohol significantly. Stillage at a level of 20% of the mash volume raised the initial rate of fermentation and the yield of alcohol but to a lesser extent than either casein hydrolyzate or urea when the latter were present in optimal concentration.

*Papain Added to Seed Yeast Cultures.* The absorption of amino nitrogen from grain mashes with resultant stimulation of yeast activity raised a question as to whether the supplementing of seed yeast media would produce an effect similar to that obtained by adding papain. Ten mg. % papain was added at the time of inoculation to clarified wort prepared by saccharifying corn mash with 20% malt. Seed yeasts were propagated for 24 hours at 30°C. in this medium and in the same medium without addition of papain. To avoid the carry-over of nutrients formed by papain the yeast from each wort was separated by centrifugation, washed, and resuspended in an equivalent volume of sterile water.

Results (Table V) show that considerable activation of the seed yeast can be effected by cultivating it in a medium treated with papain.

TABLE V

THE INFLUENCE OF ADDING PAPAIN TO THE YEAST CULTURE MEDIUM UPON THE FERMENTATION OF CORN–MALT [1] MASHES AND THE RETENTION OF ALPHA–AMYLASE

| Papain added to yeast medium | Inoculum | Weight loss in grams | | | | Final pH | Yield of alcohol Proof gal./bu. | Alpha-amylase activity units/ml.[2] | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Mg./100 ml. | Percent | 19 hr. | 27 hr. | 43 hr. | 69 hr. | | | 0 hr. | 19 hr. | 43 hr. | 69 hr. |
| None | 2 | 6.8 | 10.7 | 15.0 | 16.4 | 4.1 | 4.88 | 0.7 | 0.5 | 0[3] | 0[3] |
| 10 | 2 | 11.8 | 13.4 | 16.5 | 16.6 | 4.5 | 5.05 | 0.7 | 0.5 | 0.2 | 0.1 |
| None | 10 | 8.7 | 12.6 | 14.9 | 16.3 | 4.3 | 4.97 | | | | |
| 10 | 10 | 13.4 | 14.4 | 15.5 | 16.7 | 4.5 | 5.03 | | | | |

[1] All mashes were converted with 8% malt.
[2] Units are defined by Olson, Evans, and Dickson (6).
[3] Ten ml. of mash failed to dextrinize in 30 minutes at 20°C.

Inoculum composed of 2% of papain-treated yeast gave a more rapid fermentation rate than either 2 or 10% of untreated yeast. The final pH values were again found to be higher in the mashes inoculated with yeast cultivated in the presence of papain. Likewise the latter, in contrast to the slightly more acid control mashes contained demonstrable alpha-amylase activity throughout the fermentation period.

## Discussion

The effectiveness of certain proteolytic enzymes as a partial replacement for barley malt in grain alcohol fermentations has been demonstrated in the experiments described above. When introduced at the conversion step along with suboptimal levels of malt (5% of the grain), proteolytic enzymes accelerate the rate of fermentation and produce yields of alcohol about equivalent to those obtained with 8 to 10% malt. It was also observed that the pH during fermentation

remained slightly higher in papain-treated mashes than in mashes which received only malt.

Explanation for these effects was sought by examining the influence of proteolytic enzymes upon the rates of saccharification and fermentation. That the action of papain is confined to accelerating the rate of fermentation is shown in Fig. 1. From analyses of the free amino nitrogen contents of fermenting and non-fermenting mashes it was indicated that mashes which were converted with suboptimal levels of malt might fail to satisfy the requirements of the yeast for available nitrogen. This view was confirmed by the addition of casein hydrolyzate to similarly saccharified mashes. From the data in Tables III and IV, it is indicated that malt, even at a 10% level, fails to provide the amount of suitable nitrogen needed for a near maximum rate of fermentation. Therefore, the first effect noted upon reducing malt is a limitation of the yeast's ability to ferment available maltose, rather than inadequate saccharification as might be expected. Conversely, when metabolizable nitrogen is supplied, the activity of the yeast is markedly accelerated and bacterial activity as measured by the formation of acid seemingly is suppressed. The maintenance of a less acid reaction in turn improves the stability of malt alpha-amylase, thus favoring complete conversion.

While both casein hydrolyzate and urea are markedly active in stimulating fermentation, casein hydrolyzate is superior when the two supplements are compared on an equivalent nitrogen basis. As has been suggested by earlier workers (7, 9) this quantitative difference is probably attributable to the fact that certain amino acids satisfy specific nutritional requirements of the yeast. That these components may be absorbed in substantial amounts by culturing the seed yeast in amino acid-rich media is suggested in the present experiments. It should also be pointed out that protein hydrolyzates would vary in effectiveness according to their content of these constituents.

The lowering of alcohol yields by high levels of nitrogen supplement also is more pronounced with urea than with casein hydrolyzate. In view of the facility with which yeast cells assimilate urea and ammonia nitrogen, it is reasonable to assume that urea nitrogen is largely fixed in pyruvic and other keto acids which are formed during fermentation thus shunting these intermediates from fermentative to assimilative mechanisms. Brockmann and Stier (2) have recently expressed a concordant view and have hypothesized further that such assimilative reactions are essential to an accelerated rate of fermentation since they dissipate high energy phosphate bonds accumulating during the conversion of carbohydrates to alcohol.

While the substitution of malt was accomplished in these experiments by the addition of papain, ficin, and the enzymes from *B. subtilis* cultured in stillage, a similar result might be obtained by the use of malts with greater proteolytic activity or by the supplementation of seed yeast media with available nitrogen.

**Acknowledgment**

The authors wish to express their appreciation to Mr. R. G. Dworschack for determinations of alpha-amylase activity.

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# FERMENTATION CABINET FOR 100-GRAM DOUGHS[1]

H. E. RASMUSSEN and J. ANSEL ANDERSON

**ABSTRACT**

A square cabinet (38×54×32 inches) encloses an upright cylinder in which doughs are held on five circular shelves attached to a common shaft and rotated at 3 r.p.m. by a friction drive mounted below the cabinet. Air is conditioned beneath the cylinder and is circulated, by a centrifugal blower, up through the perforated shelves and down between cylinder and outer walls. A dry-bulb thermoregulator controls temperature at 86°±0.08°F. by operating heating lamps installed between the cylinder and outer walls. A wet-bulb thermoregulator, in front of the blower inlet, controls humidity at 90±1% by operating a Bahnson-type humidifier inserted through the bottom of the cabinet. Rubber mounts and external location for all motors minimize vibration and heat input. The cabinet is made almost entirely of aluminum and is rustproof throughout.

A fermentation and proofing cabinet, with precise control of temperature and humidity, has been constructed to accommodate 100-g. doughs for a continuous baking schedule in which doughs are mixed at 5-minute intervals and proofed to time. Although at least two cabinets for test baking have previously been described (1, 2), and several types can be obtained commercially on the continent, certain features of the new cabinet seem to merit description because of their wide application in designing laboratory cabinets for various purposes. Accordingly, a general description of the cabinet, detailed descriptions of principal parts, and information on operation are given in the following three sections.

## General Description

Fig. 1 shows a photograph of the equipment. The aluminum cabinet (38×54×32 inches) encloses an upright cylinder in which dough containers are held on five circular shelves attached to a common shaft. Air is conditioned below the cylinder and circulated up through the perforated shelves and down between cylinder and outer walls. Horizontal rotation of shelves and vertical circulation of air insure uniform conditions for all doughs. The cabinet stands on a welded angle-iron frame, 21 inches high, enclosed with plywood panels and doors.

Fig. 2 illustrates the design with an exploded view of the cabinet. The shelf assembly, which is shown partially withdrawn, drops into the surrounding cylinder, to which the bearing spider is then bolted. The cover fits over the casing and is secured by screws.

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At each of the front corners of the cabinet a long inspection door gives access to the air-conditioning chamber and to a cavity which houses sockets and wiring for heating bulbs. A cutaway portion of the insulated double wall shows the position of the bulbs.

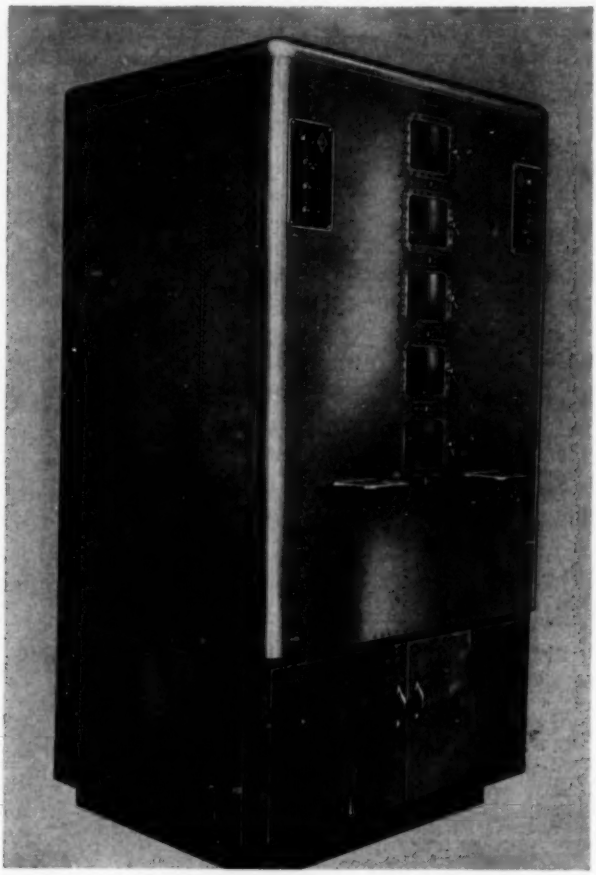

Fig. 1. Photograph of fermentation and proofing cabinet.

The horizontal partition, with four triangular holes for downward air flow and two circular holes for upward flow, fits within the casing against the bottom of the cylinder and is secured by rivets. When the base plate and blower compartment are riveted in place, an enclosed duct for air distribution and a re-entrant cavity for the blower are formed. The wet-bulb thermoregulator and its reservoir (on a small stand which is not shown) are placed at the left against the blower compartment with the wick in the direct draft; the dry-bulb thermoregulator is attached to the compartment on the right.

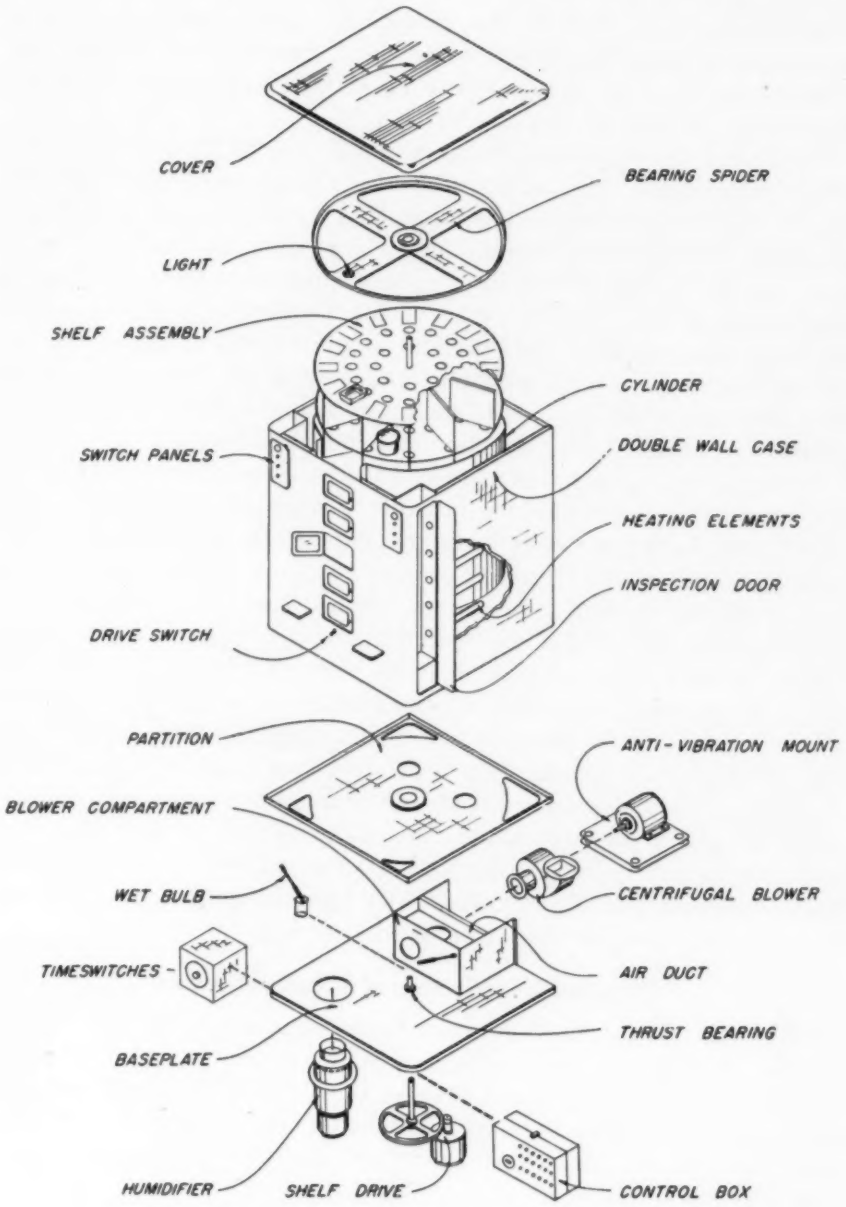


Fig. 2. Drawing of exploded view of cabinet.

The upper part of the humidifier is inserted into the conditioning chamber through a hole in the base plate. The lower part of the humidifier, a box containing time-switches, the drive for the shelves, and a control box containing electrical accessories are housed within an enclosed stand beneath the cabinet (Fig. 1).

Dry- and wet-bulb temperatures of 86° and 83.5°F., giving a relative humidity of 90%, are maintained in the cabinet. Variations of these conditions with time are minimized by using mercury-in-glass thermoregulators sensitive to ±0.05°F., by placing the wet bulb directly in front of the blower inlet, and by adjusting rates of heat and vapor inputs to give short control cycles with little overrun. Variations in space are minimized by rapid vertical circulation of the air assisted by the mixing action of the rotating shelf assembly. Moreover, small doors and the shielding effect of shelf partitions minimize variations in cabinet conditions during insertion or removal of doughs.

Four general features of the design deserve mention. Firstly, as the temperature of the baking laboratory is maintained at 78°F. and rises higher for a few weeks during the summer, precise control of the cabinet at only a few degrees above room temperature requires that heat input be reduced by external mounting of drive, blower, and humidifier motors, and of the heated humidifier reservoir. Secondly, vibrations that might affect doughs are prevented by ball bearings for the shelf assembly, a drive that starts and stops smoothly, and anti-vibration mounts for all motors. Thirdly, all working parts of the equipment are readily accessible for servicing or eventual replacement. And fourthly, as the baker stands in the same place to insert and remove doughs on all shelves, the cabinet can be conveniently placed to minimize steps required during the baking schedule. In short, though the cabinet is relatively complex, it has been designed to provide convenient operation and long, trouble-free service.

## Detailed Descriptions of Principal Parts

The following subsections describe the main details of construction of the cabinet and of the various auxiliary parts: drive, humidifier, blower, controls, wiring, and time switches. The complete unit was designed in this laboratory. Sheet metal work was done by a Winnipeg firm, but all other construction and all installations were undertaken in the laboratory.

*Construction.* Except for the base plate, the cabinet is constructed entirely of $\frac{1}{16}$-inch aluminum plate (3S$\frac{1}{2}$H) with all joints riveted. The base plate consists of a $\frac{1}{4}$-inch aluminum plate supported on an angle-iron frame with channel-iron cross braces. A vertical thrust bearing at the center of the base plate carries the full weight of the shelf assem-

bly. The shaft, which is $\frac{3}{4}$-inch stainless steel, is also supported above and below the cylinder by horizontal thrust bearings. Sealed ball bearings, glass-wool insulation, and rustproof materials for the cabinet and all auxiliary parts minimize the injurious effects of high humidity.

*Drive.* Rotation at about 3 r.p.m. was required for the shelves. An ordinary motor and reducing gears would start the shelf with an undesirable jerk and would require a relatively complex clutch mechanism for bringing selected dough containers into position for removal. These difficulties were overcome by a friction drive and a disc induction motor (G.E. type AZ-133A) such as is used for turning record tables in broadcasting stations. The motor has a built-in governor which per-

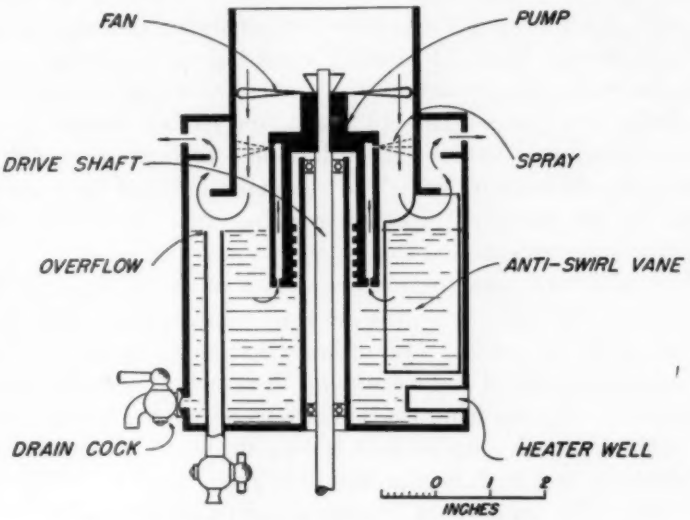


Fig. 3. Cross-section drawing showing the design of the humidifier.

mits the speed to be adjusted to 65 r.p.m. A No. 6 stopper on the motor shaft rotates the shelf assembly by driving against the rim of a 12-inch pulley attached to the shelf shaft below the bottom of the cabinet. Two Lord mounts serve as hingelike supports for the motor and allow its weight to hold the stopper against the pulley (Figs. 2 and 4). This drive permits the baker to swing the shelves by hand, either forward or backward, to bring any crock into position for removal within a second or two. In addition, the motor is silent, runs with minimum vibration, and starts and stops the shelf slowly and smoothly.

*Humidifier.* Fig. 3 shows a drawing of the humidifier. It is of the Bahnson type but with the design modified so that the motor is mounted beneath rather than above the unit. This change involved construction of a reservoir with a central bearing housing extending

above the water, and of a modified pump running on the outside of this housing.

Rotation causes four slanted openings in the bottom of the pump to drive water up between the sleeves and out of four pin-holes near the top to form a horizontal curtain of fine spray in the chimney. The fan at the top of the chimney blows air down through the spray, over the surface of the water, and out through openings around the top of the reservoir. Water is prevented from being thrown up between the pump and bearing housing by a reverse thread cut on the inside of the pump at the bottom.

Efficient humidification requires that the water be maintained 10° to 15°F. above the temperature of the cabinet. A well is therefore provided to accommodate a constant heater consisting of a standard 20-w. tip for a soldering iron. The unit is self-balancing; if the operating time of the humidifier increases, the greater heat loss through evaporation causes the temperature of the water to fall slightly, and vice versa; equilibrium is attained when heat loss through evaporation and radiation equals heat input. The reservoir is filled by opening the stopcock on the overflow pipe and pouring water down the chimney until a few drops overflow.

Other details of construction are shown in the drawing: baffles to remove entrained water from the air stream; antiswirl vanes; overflow pipe; and drainage cock. The motor (Bodine, type NSE-11R, 1/40 h. p.) is mounted with Lord mounts on three columns on the bottom of the reservoir. By lowering the voltage to 85 with a transformer, the speed of the motor is reduced to 3,500 r.p.m., so that the unit will run almost silently and with negligible vibration.

*Blower.* The centrifugal blower consists of a standard rotor, 3 inches wide by 5 inches diameter, driven at 1,725 r.p.m. by a $\frac{1}{8}$-h.p. motor, and delivering approximately 40 c.f.m. In order to prevent transmission of vibration by avoiding direct contact between blower and cabinet, a special rotor housing was constructed (brass, nickel plated) to provide a collar and flange at the inlet and a flanged outlet (see Fig. 2). When the unit is in place, inlet and outlet flanges are centered within larger holes in the blower compartment, and the annular spaces are closed by rings of $\frac{1}{4}$-inch rubber sealed against flanges and compartment by suitable clamping rings. The motor, with rotor housing attached to it, is supported on four pairs of Lord mounts which effectively damp both vertical and transverse vibrations. Fig. 4, a photograph of the back of the cabinet, shows the assembly. The transformer on the left decreases the running temperature of the motor by reducing voltage to 90, and the small fan on the back of the motor keeps the temperature down within the cavity.

*Controls.* The thermoregulators (8-inch, Aminco No. 4-201) actuate battery-operated relays (Kurman, No. 190036). Three 40-w. showcase lamps, 12 inches long, on each side of the cabinet are controlled by the dry-bulb thermoregulator. Two lamps in series are connected in parallel with the third, so that 60 w. are provided on each side. Four additional 40-w. bulbs provide stand-by service for more rapid heating of the cabinet.

The wet-bulb thermoregulator controls the humidifier. When the wet-bulb temperature falls and breaks the contact in the thermoregu-

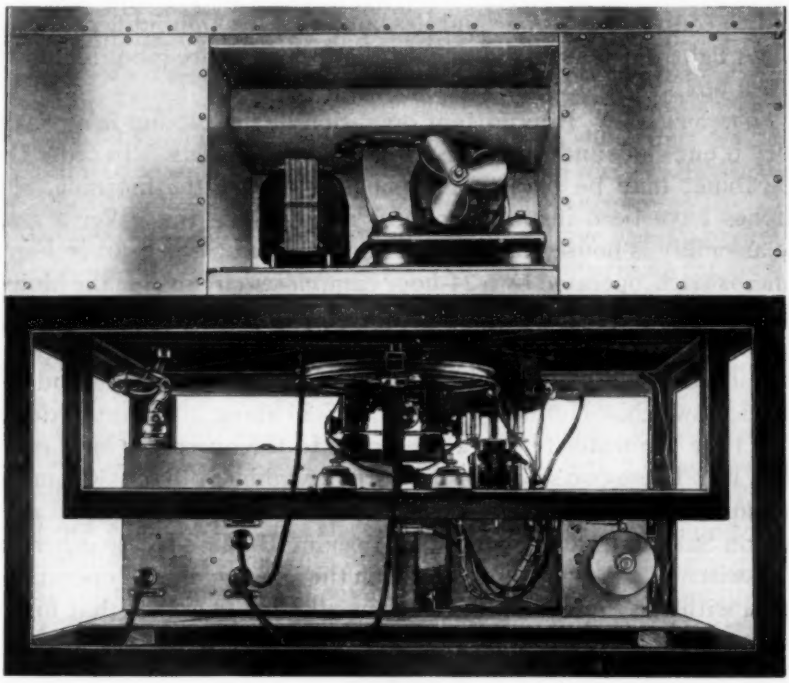

Fig. 4. Rear view of cabinet with panels removed from stand.

lator, the humidifier motor starts and runs until contact is re-established. As the cabinet is not completely airtight, humidity tends to fall when the humidifier stops through interchange of air with the room. Sensitive operation is insured by locating the wet bulb immediately in front of the blower inlet. The thermoregulator and its reservoir (100-ml. beaker) are mounted on a small stand which can be readily removed through the left-hand inspection door for servicing; this involves rinsing the sock and filling the reservoir.

*Wiring.* As the cabinet operates at high humidity, most of the wiring and accessory electrical equipment is housed in a separate con-

trol box beneath the cabinet. This is shown at the left in Fig. 4. The box contains the thermoregulator relays and their batteries, fuses for each circuit, and certain auxiliary switches. Leads from within the cabinet and from the time switches are brought into the box by Cannon connectors; and individual outlets are provided for plugging in the main lead supplying 110-volt current, the three motors, and the humidifier heater.

The principal switches are mounted on the face of the cabinet, the drive switch below the doors, and the others on panels near the top corners (Fig. 1). In addition to the master switch, three others are provided to control the blower and heating units, the humidifier, and the cabinet light. Two switches for auxiliary heaters are mounted on the control box.

*Time Switches.* Intermittent operation of the cabinet is advisable to dry it out daily and to reduce wear on working parts. In order that the cabinet may be ready for immediate use in the morning, time switches have been installed to turn it on before the baker arrives. The assembly is housed in the metal box shown on the right in Fig. 4. A microswitch, operated by a 24-hour cam, closes circuits for the blower, controlled heaters, and humidifier heater at 4:30 a.m. By 8:00 a.m., the cabinet temperature is controlled at 86°F. and the humidifier water has reached 105°F.; the humidifier is then turned on by a second cam and microswitch. Humidity rises to 90% in about 10 minutes, during which time the water temperature drops to the operating level of 98° to 100°F. These two switches also turn the cabinet off at 4:30 p.m. A third microswitch, with a 7-day cam, prevents the cabinet from operating on Saturdays and Sundays by opening the line to the other two microswitches. A submaster switch on the panel, marked "operation," is used with the time switches; it opens all circuits except that for the clock motor.

## Operation

Although the cabinet is relatively complex, its operation is simple. During the week, the baker has only to move the "operation" switch when he decides whether or not he will need the cabinet next day. In addition, he must service the wet-bulb thermoregulator and fill the humidifier, which takes about 3 minutes.

The dry-bulb and wet-bulb thermoregulators are sensitive to less than ±0.05°F., are accurately set at 86° and 83.5°F., and maintain their settings indefinitely. Because of slight overshooting of the control level when heat is added, the variation in dry-bulb temperature is ±0.08°F. Equipment was not available for measuring the corresponding variation in the wet-bulb temperature, but this is probably of the same order.

As the thermoregulators operate independently, one may be at its maximum while the other is at its minimum and vice versa; accordingly, the spread between dry- and wet-bulb temperatures probably varies between 2.34° and 2.66°F., which is equivalent to a variation of less than ±1% in relative humidity. But operational cycles are short; for heaters, 3 minutes on and 11 minutes off; and for the humidifier, 0.5 minute on and 5 minutes off. Malloch (3) has demonstrated that doughs cannot pick up such rapid variations in temperature, and observation shows that this is true of humidity variations also. Thus, for all practical purposes, the cabinet may be said to operate at absolutely uniform temperature and relative humidity in time.

Since the doughs rotate horizontally, effective variations of conditions in space are restricted to those that occur between shelves. Observations made with precise thermometers show that the rapid vertical circulation of air reduces the difference in temperature between top and bottom shelves to about 0.1°F. This is negligible.

Data on the reproducibility of loaf volumes have been compared for the new cabinet and its predecessor. Direct comparisons could not be made because expediency required that the old cabinet be retired to make room for the new one. Unfortunately, indirect comparisons are less satisfactory because reproducibility varies with both handling properties and loaf volume, though the effect of the latter can be partially offset by calculating coefficients of variation. The last six substantial investigations made with the old cabinet gave coefficients, arranged in increasing rather than in chronological order, of 1.4, 1.5, 1.5, 1.6, 1.8, and 2.1%; and the two large investigations made with the new cabinet gave coefficients of 1.2 and 1.4%. Accordingly, there is some indication, though no convincing proof as yet, that reproducibility has improved. Irrespective of whether this hypothesis is substantiated, the convenient operation, easy servicing, and durability of the new cabinet suggest that the cost of engineering and constructing it will prove to be a sound investment.

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# RELATIONSHIP OF DIASTATIC ACTIVITY TO FLOUR STRENGTH [1]

C. C. WALDEN [2] and R. K. LARMOUR [3]

**ABSTRACT**

Two types of gassing rate curves, due to the fermentation of maltose produced by flour diastatic action, were observed.

Autolytic maltose value and protein content of flours are related inversely.

With baking formulas capable of maintaining adequate gassing rates to oven time, differentiation between flours of varying strength occurs mainly in the oven, rather than during the proof period. The practice of proofing to constant height should not be followed in evaluating total flour strength.

The autolytic determination of the diastatic enzymes of flour devised by Rumsey (10) and further refined by Malloch (8) and Blish and Sandstedt (3) provides the method now widely used to determine the maltose-producing ability of flour. The maltose formed may be estimated by any conventional physical or chemical method applicable to sugars.

The manometric method (5), which parallels dough conditions, measures the pressure developed by the fermentation of the sugars produced in the autolytic system. The degree of relationship between this method and the autolytic maltose value is high, although the latter does not include the preformed sucrose in the flour. Gas production may be measured volumetrically (2), this method permitting a ready evaluation of gassing rates.

The current concept of the autolytic production of flour maltose is that of an activity of excess beta amylase under limiting substrate conditions. Following the observation of Alsberg and Griffing (1) on the effect of fine grinding in increasing maltose production, various workers have studied the effect of starch injury on autolytic diastatic activity. Dadswell and Wragge (6) concluded that diastatic activity is correlated with starch injury resulting from milling, and Jones (7) determined that the maltose figure is dependent on the content of damaged starch granules. In the absence of diastatic supplements, the maltose figure is a measure not of the beta amylase but of the degree of mechanical injury occurring to the starch granules during the milling process.

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Paper No. 259 of the Grain Research Committee of Canada.

[2] Chemist, the Quaker Oats Company of Canada Limited, Saskatoon, Saskatchewan; formerly part-time employee and graduate student at the University of Saskatchewan.

[3] Director of Research, Maple Leaf Milling Company Ltd., Toronto, Ontario; formerly professor of chemistry at the University of Saskatchewan.

Munz and Bailey (9) observed that the higher the maltose value of a given flour, the longer was its effective gassing period, and that additions of sucrose eliminated these differences between flours. Walden and Larmour (13) have shown that a high yeast, high sugar, high salt formula is necessary if the baking data are to reflect strength of the flour rather than the limitations of formula. This paper deals with further work on the relationship between the maltose-producing ability of varying flours and their behavior with the above type of formula.

## Materials and Methods

The series of flours used consisted of 96 flours, 16 each from the following bread wheat varieties—Marquis, Thatcher, Apex, Renown, Regent, and Rival. The protein variation for the entire series was from 7.9% to 18.6% (13.5% moisture basis) with a variation of the same order within each variety. As the inclusion of all the data obtained with these six series would encumber this report unduly, only a few typical examples will be presented. The complete original data are on record (Walden, 12).

Natural gassing rate curves are those of 3% yeast, flour, water doughs. Baking data and gas retention measurements were obtained with the following formula: 3% yeast, 1.75% salt, 5% sugar, 4% nonfat milk solids, 3% shortening, 0.1% ammonium dihydrogen phosphate, 0.3% 20° Lintner malt, 0.001% potassium bromate. Pup loaves from 100 g. doughs were mixed, fermented, proofed to constant time, and baked according to the A.A.C.C. procedure. Gassing rates and gas retention were measured as outlined by Walden and Larmour (13). Maltose values were determined by the method of Blish and Sandstedt (3).

## Results

*Baking Data.* Using the above formula, which provides an adequate rate of gassing through the fermentation and pan-proof periods and into the oven period (13), baking tests were made on the above series of flours. The statistical data are summarized in Table I. The correlations between protein content and loaf volume indicate that where gassing rates are adequate, there are few remaining variables influencing the relationship of loaf volume to gluten quantity. Variation in gluten quality within this series appears to be small.

*Natural Gassing Power.* Thirty-two flours of the original series, comprising all the Marquis and Thatcher samples, predominant Western Canadian varieties, were examined for natural gassing power. Fig. 1 reproduces the gassing rate curves of a Marquis subseries and illustrates the various phenomena encountered in all the flours studied.

TABLE I

STATISTICAL CONSTANTS FOR LOAF VOLUME AND PROTEIN CONTENT

| | Entire series | Apex | Marquis | Rival | Renown | Regent | Thatcher |
|---|---|---|---|---|---|---|---|
| Mean protein of flour $\bar{x}$ | 13.88 | 13.96 | 13.46 | 13.58 | 14.05 | 14.14 | 14.09 |
| Mean loaf volume $\bar{y}$ | 923.4 | 905.2 | 908.5 | 901.3 | 930.1 | 935.3 | 959.8 |
| Correlation coefficient $r_{xy}$ | +0.971 | +0.991 | +0.979 | +0.982 | +0.988 | +0.994 | +0.963 |
| Regression coefficient $b_{yx}$ | +46.27 | +37.08 | +40.88 | +52.64 | +42.54 | +46.04 | +54.34 |
| 1% point | 0.254 | 0.623 | | | | | |

All eight flours exhibit double maxima gassing curves, with a small variation in the first maximum in respect to height and time, and a pronounced differentiation in the second maximum. Walden and Larmour (13) concluded that the first maximum can be explained by

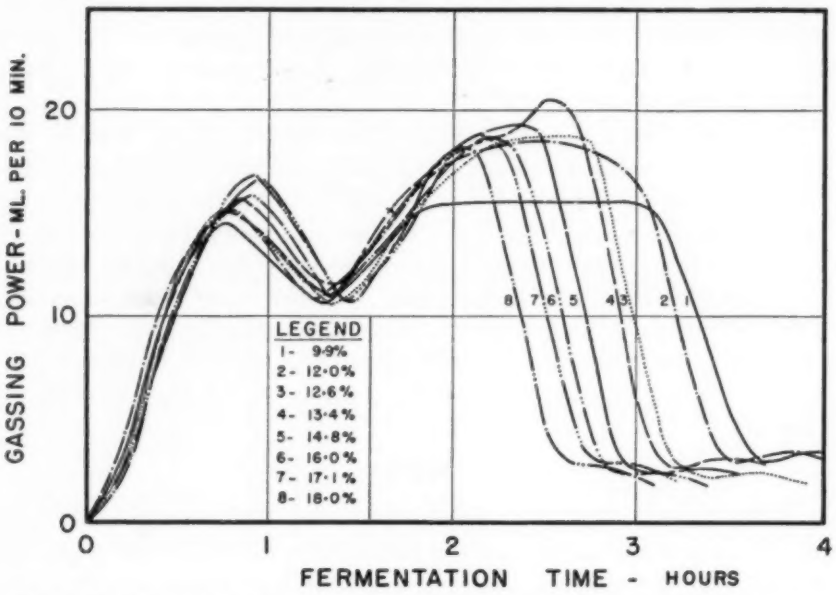


Fig. 1. Gassing rates of composite Marquis wheat flour doughs fermenting with 3% yeast only. Protein contents shown in legend.

the assumption that the initial fermentation uses the sucrose present in the flour, and when this becomes a limiting factor the rate falls off until the fermentation of maltose is initiated, whereupon the rate of fermentation accelerates again to produce the second maximum. The height and duration of the second maximum is directly related to the maltose-producing capacity of the flour. It is interesting to note in Fig. 1 that although there is some displacement of the first maximum, indicating that slight differences of sucrose content of the flours existed,

all the minima occurred at practically the same rate and time. Thus when the second acceleration of fermentation occurred, all the flours started on practically an equal basis, and the real differentiation in their gassing powers is evident in the latter part of the curves.

The two types of second maxima are indicative of the variations observed in all the flours studied. Above 10% protein the second maxima reach a sharp peak and then decline in order of descending protein content. At levels of 10% protein or lower, a flat extended maximum, as illustrated by curve 1, Fig. 1, was obtained. This plateau-type curve does not reach the maximum value shown by higher protein flours, but extends over an appreciably longer period. Without added baking supplements only the lower-protein flours could make any appreciable contribution to gassing during the pan-proof period (between 3rd and 4th hours according to A.A.C.C. procedure).

The data presented in Table I show that with a proper formula which will maintain the gassing rate, there is a very high correlation between protein and strength as indicated by loaf volume. It is obvious from Fig. 1 that unless adequate sugar, or diastatic supplement, or both are provided in the formula, the high-protein flours will be penalized unduly and that the low-protein flours will appear relatively much stronger than they really are.

While a lack of ability to maintain an adequate gassing rate through the fermentation period might be deemed a quality factor in a finished commercial flour, it ought not to be so regarded in estimating inherent strength of wheats because it can be readily changed either by additions to the flour itself or to the baking formula. In brief, gassing power is a function of the baking formula rather than of the quality of the wheat or flour.

*Correlation of Maltose Figure and Protein Content.* The entire series of 96 flours was analyzed for autolytic maltose value and the data, shown graphically in Fig. 2, reveal clearly the inverse relationship with protein content. The statistical constants for maltose value and protein content are as follows:

| | |
|---|---|
| Mean protein of flour $\bar{x}$ | 13.88 |
| Mean maltose value $\bar{y}$ | 188.19 |
| Correlation coefficient $r_{xy}$ | −0.724 |
| Regression coefficient $b_{yx}$ | −9.074 |
| 1% point | 0.254 |

The correlation coefficient, −0.724, although not high, is significant.

This explains in part the extended gassing power of the lower-protein flours: they produce more maltose. But it fails to explain why

the rate of gassing, although more extended, is never as high as in the high-protein flours.

*Gas Retention.* Loaf volume is the sum of the dough volume immediately after panning (virtually a constant) and the volume of gas retained during the pan-proof and oven periods. Where gas production has been eliminated as a variable, the remaining variable is the ability of the dough to retain the gas. Having developed such condi-

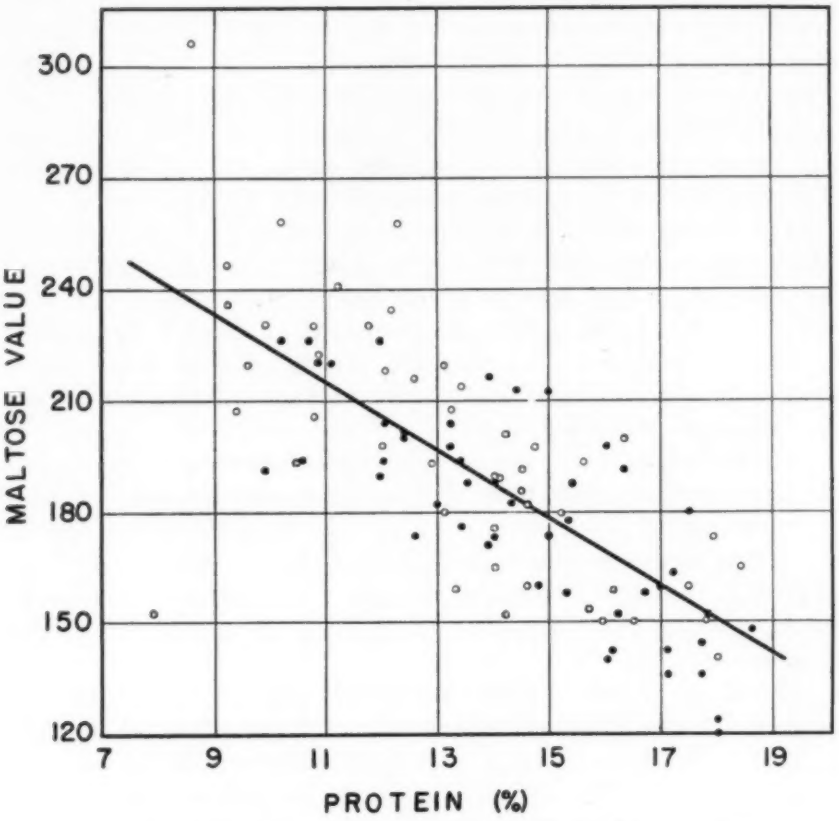


Fig. 2. Scatter diagram showing relation between maltose value and protein content.

tions, it is possible to evaluate the role of the pan-proof and oven period in gas retention.

Proof height is frequently used to indicate differences in proofing ability. A more accurate, but not routine, measurement of this property is a volumetric measurement of dough volume increases. Gas retention measurements on the high yeast, high sugar, high salt formula, given graphically in Fig. 3, are for those flours whose natural gassing rate curves are shown in Fig. 1. The left-hand group of curves

gives dough volume increases from time of mixing to the beginning of the pan-proof period. At the beginning of the pan-proof period, measurements were continued on fresh dough aliquots which had been fermented and punched since mixing time according to the A.A.C.C.

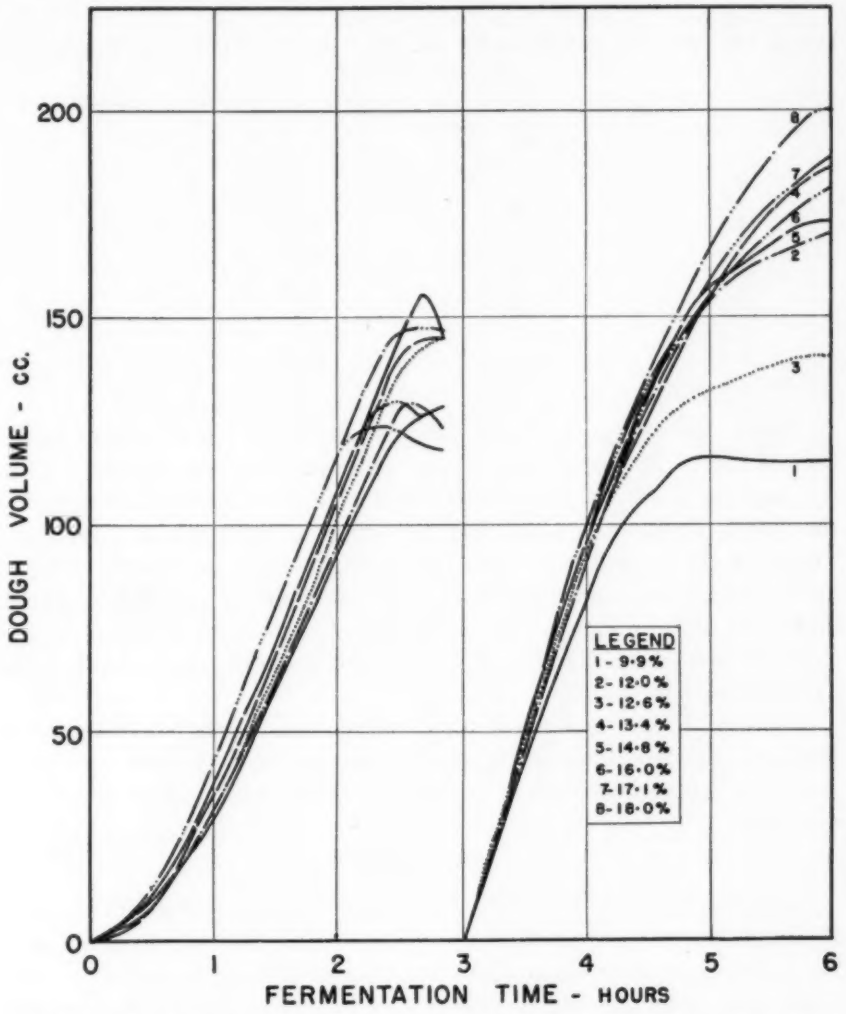


Fig. 3. Dough volume changes of composite Marquis wheat flour doughs fermenting on baking formula.
Left-hand group of curves—from time of mixing the dough.
Right-hand group of curves—from beginning of pan-proof period.

procedure. Dough volume increases for these doughs from 3 hours to 3 hours and 55 minutes correspond to pan-proof increases.

Dough volume increases to the end of the pan-proof period on the basis of 100 g. flour are shown in column 2 of Table II. These values

are smaller than the actual dough volumes at oven time by the volume of the dough after panning. Correspondingly the oven springs, obtained by subtracting the dough volume increases from the loaf volumes, are too great by this quantity.

TABLE II

DOUGH VOLUME (PAN PROOF) INCREASES, LOAF VOLUMES, AND OVEN SPRINGS FOR MARQUIS SERIES

| Protein content | Dough volume increase (basis 100 g. flour) | Loaf volume | Oven spring |
|---|---|---|---|
| % | *cc.* | *cc.* | *cc.* |
| 9.9 | 300 | 776 | 476 |
| 12.0 | 332 | 846 | 514 |
| 12.6 | 342 | 832 | 490 |
| 13.4 | 338 | 894 | 556 |
| 14.8 | 350 | 961 | 611 |
| 16.0 | 346 | 1019 | 673 |
| 17.1 | 352 | 1055 | 703 |
| 18.0 | 362 | 1114 | 752 |

The range in dough volumes corresponding to a loaf volume variation of 338 cc. is only 62 cc. Even assuming that the gas retained in the pan-proof period expands thermally to oven temperature within the dough before crust formation, this range is increased only to 104 cc. This is a theoretical limit only, such conditions never being reached under actual baking conditions. The oven spring parallels loaf volume, and differentiation between flours on this basis is much greater than obtained with dough volumes. Similar results were obtained on 24 additional flours (12).

Differentiation between flours of varying strength, baked by a rich formula, occurs mainly in the oven, and the dough volume, which in actuality is a more accurate measure of proof height, is of minor significance.

## Discussion

The longer period of effective gassing by low protein flours is due to their greater maltose-producing ability. With the current concept of the autolytic maltose figure, this must be attributed to a greater total content of mechanically damaged starch. From their lower protein content it follows that these flours possess a higher total starch content, although milling damage is normally associated with kernel vitreousness or high protein content.

Walden and Larmour (13) have shown that with a high yeast, high salt, high sugar formula the fermentation of autolytic flour maltose is retarded to the point where it may be of little significance under the A.A.C.C. baking procedure. The foregoing evidence shows that if

maltose fermentation is a contributing factor before crust formation, it is not a critical one: gas production has been eliminated as a variable and the doughs, having adequate gas production during the pan-proof and oven periods, have retained the gas to their maximum ability to do so. As a result, baking data show a high correlation between loaf volume and protein content. Under such conditions, the authors believe that the resulting loaf volume reflects fundamental flour strength.

The laboratory practice of proofing to constant height was recommended to equalize differences in "Factor M" content between flours (11). "Factor M" is a biocatalytic activator, specific to maltose fermentation, the existence of which was postulated by Blish and Sandstedt (4). Since it has been shown that neither maltose production nor maltose fermentation in a dough have any critical influence on baking strength with rich formulas, the necessity of proofing to constant height is obviated. Further studies on this type of formula have indicated that the major differentiation between flours occurs not during the proof period, but in the oven. There is a tendency for differentiation of flour strength during the pan-proof period, but it is not reliable. Nevertheless the authors maintain that the minor contribution pan-proofing makes to loaf volumes under adequate gassing conditions is due to differences in flour strength and any attempt made to equalize proofing rates will mask these differences.

In commercial baking with lean formulas, proofing to constant height may be of value in maintaining a uniform product. Experimental baking, designed to imitate commercial practice, may also find proofing to height of some value.

But in other phases of the milling and baking industries, where the volume-producing potentialities of a flour are of utmost importance, proofing to height has no place in the baking procedure.

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# MICROSCOPICAL OBSERVATIONS AND PROCEDURES USED IN A STUDY OF INDUSTRIAL CORN STEEPING [1]

JOHN A. WAGONER [2]

## ABSTRACT

A microscopical study was made of the protein matrix and the cellulose walls of the corn endosperm while the grain proceeded through the usual industrial wet milling steeping process. Cross sections of the steeped grain were made with a freezing microtome. Starch was cleared with chloral hydrate; protein and cellulose were differentially stained with thionine. The method gave rapid, reproducible results in which the occurrence of small changes could be distinguished microscopically.

The lactic acid phase of the steeping process softened the corn by the absorption of water. In the later portion of the process the sulfurous acid concentration was high and the protein matrix was disrupted, leaving the protein massed against the cellulose cell walls. Additional steeping loosened this protein from the cellulose walls.

Corn stored a year was steeped along with some freshly harvested grain, and it was shown that the protein network of the new corn was disrupted more rapidly. This was not caused by a difference in the moisture content, since both samples attained the same moisture content early in the steeping.

In the wet milling process for the manufacture of corn starch, there is considerable plant readjustment in the fall of the year when the new corn is started through the process. It has been observed over a period of years that if sound new corn is steeped for a shorter length of time and the concentration of the sulfurous acid reduced, it could usually be processed in about the same manner as the old. However, since this is not always the case, a microscopical study was started to ascertain the structure of a well-steeped kernel of corn and the differences between old and new corn during the processing.

Kerr (2) describes a number of methods of commercial processing of corn. Practically all of the wet milling industries use the counter

[1] Manuscript received May 25, 1948. Presented at the Annual Meeting, May, 1948.
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current method, or a modification thereof, in which a sulfurous acid solution (about 0.25%) is added to the almost fully steeped corn. This solution is then pumped out of the bottom of that steeping tank to the tank in which corn has been steeping for a slightly shorter length of time. This procedure is continued throughout a battery of steeps so that the solution placed on the dry corn has been transferred through the line of steeping tanks. As the solution is circulated, the sulfurous acid is absorbed by the corn so that its acidity is reduced from 0.25% to 0.02%. Thus the most completely steeped corn is processed in essentially a sulfurous acid steep. Most of the sulfurous acid has been absorbed during the first 10–12 hours, leaving conditions which favor the production of lactic acid by fermentation. Thus as the steeping is continued, the water pumped on the corn which first enters the process has a high lactic acid, and a low sulfurous acid, content. During the whole cycle the procedure has been regulated so that these two acids counterbalance to keep the system within a pH range of 3.5 to 4.5. Most of the industries steep their corn from 36 to 50 hours at temperatures from 115° to 130°F.

Studies of microscopic examinations of corn during steeping have been published by Cox, MacMasters, and Hilbert (1). However, their steepings were for shorter periods of time, and the corn was steeped immediately in the most concentrated sulfurous acid solution without the lactic acid steep encountered in industrial practice. Thus, while it was decided to use essentially their methods of sectioning, the products investigated had been subjected to quite different treatment.

## Materials and Methods

*Corn Samples.* Since very little was known about the actual progress of steeping within a kernel of corn, samples of corn were removed from the steeping tanks at all stages in the steeping cycle. Each sample of corn was sectioned, stained, and mounted within an hour after the sample was removed from the tanks. In this manner progressive changes were followed on corn which was considered to be processing normally. Later, when new-crop corn was received, a study of comparative steeping of old and new corn was made using as receptacle a wire basket divided in the center and containing about a pound of each. The charged basket was lowered into a steeping tank as soon as the tank had been filled, and at 4-hour intervals samples of about 25 g. of each were removed for study.

*Moisture Analysis.* The sample was rinsed immediately to remove the adhered solubles, and the surface moisture blotted. Its moisture content was determined by drying to constant weight in an oven maintained at 110°C.

*Microscopic Examination.* Several kernels of average shape and size were sectioned, using a Spencer sliding microtome. First a cross-sectional cut was made about halfway between the upper end of the scutellum and the crown; another was cut about 3 mm. closer to the tip. This center section was placed upon the object holder in the freezing chamber and mounted in a 10% solution of a water-soluble

Fig. 1. Cross section of old corn steeped 12 hours. This section is from the middle of the horny endosperm, midway between the tip and the crown. The protein matrix is intact, and the cellulose walls are very strong. × 430.

gum, such as gum arabic or talha. By using this gum solution, the sections could be obtained with very little tearing of the pericarp from the endosperm. The section was frozen by carbon dioxide gas, care being taken to encase the sample completely with the gum solution. Freezing was continued for about 3 minutes by which time solidification was complete. Sections 40$\mu$ in thickness were cut, removed from the blade with a moistened camel's hair brush, and placed in distilled water. As soon as several good sections were obtained, one of them

was placed on a microscope slide and a drop of 0.05 *N* iodine solution placed on it. The iodine was allowed to stand 3 or 4 minutes and washed off. Then several drops of 75% chloral hydrate were added to the section, and after 30 seconds or so the starch granules could be brushed out of the section with a fine-hair brush. With these samples the chloral hydrate proved more satisfactory than the use of sulfuric

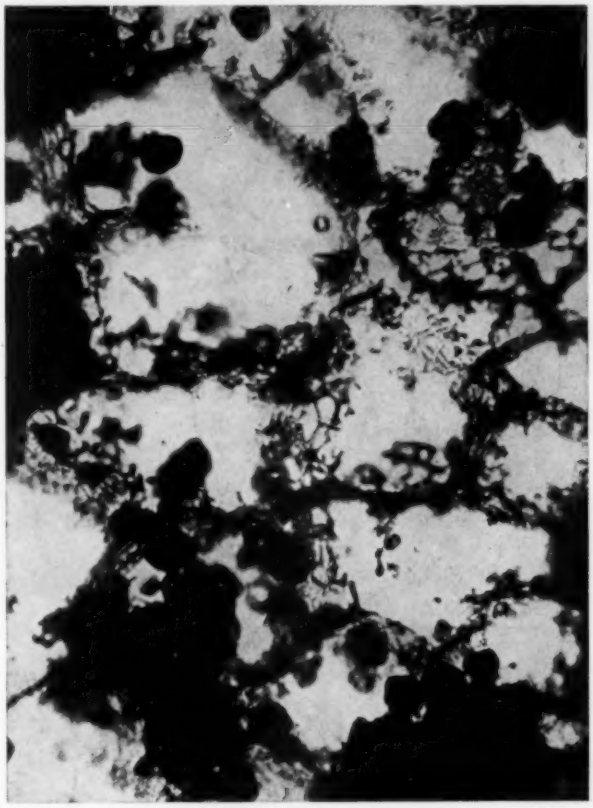

Fig. 2. Cross section of old corn steeped 36 hours, the section taken from same area as Fig. 1. The protein matrix is disrupted far into the horny endosperm, but the cell walls are still strong. × 430.

acid described by Cox, MacMasters, and Hilbert (1). The completely steeped sections were very fragile, and an extra second or so of treatment with the acid would destroy the section, but a variation in treatment time of 30 seconds with the chloral hydrate had no appreciable effect. When light to moderately steeped grains were sectioned, either method gave good results. The section was then washed, and a few drops of 0.25% thionine solution, adjusted to a purple color with ammonia, were placed on the section. After it was allowed to stand 10 minutes, this solution was rinsed off. The protein was stained

yellow, the cellulose blue to reddish, and the starch remaining in the section appeared black. This section can be mounted in water if the sections are to be photographed immediately, but a temporary mount of a solution of polyvinyl alcohol will make the slide usable for several days.

## Results and Discussion

Fig. 1 shows a section taken from a matured corn grain with moderately low moisture content which had been steeped for 12 hours.

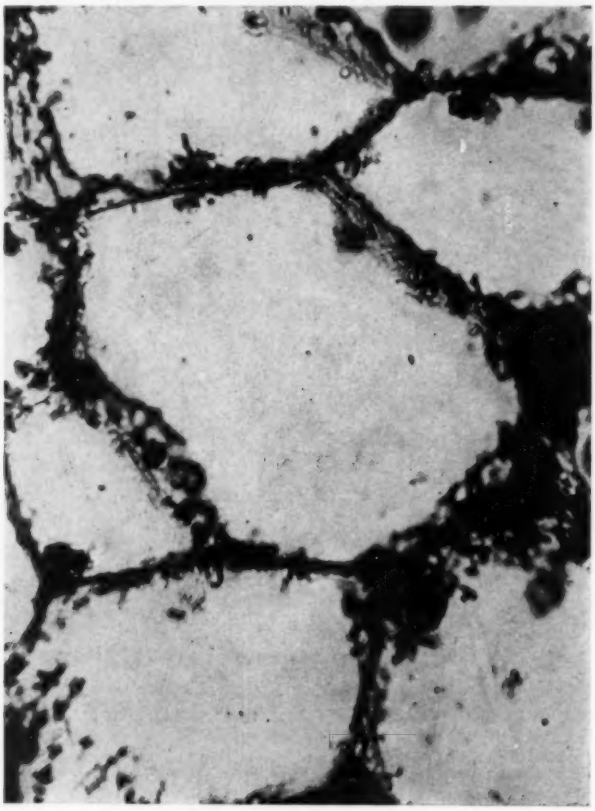

Fig. 3. Cross section of old corn steeped 50 hours, the section taken from the same position as Figs. 1 and 2. The protein matrix is disrupted out to the aleurone layer; the protein is folded against the cell walls; and the walls are quite fragile. × 430.

Up to this time the corn was very hard and sections were difficult to make. The protein matrix is still intact, the starch is held tenaciously, and the cellulose walls are very firm. Only slight changes can be noticed in any of the sections until the corn has been steeped for about 24 hours. At this time the sulfurous acid content of the steepwater is beginning to rise quite rapidly, and the protein matrix in the floury

endosperm begins to break up, freeing the large starch granules. At 36 hours the protein network has dispersed through most of the horny endosperm as shown in Fig. 2. Continued steeping furthers this disorganization until the protein network has disappeared even around the small granules next to the aleurone layer. A well-steeped kernel of corn is illustrated in Fig. 3. This corn had been steeped for 50 hours, and it is noticed that there is no protein matrix, but the cellulose cell

Fig. 4. Cross section of new corn steeped 36 hours. The section was taken in the same position as Fig. 2. The protein matrix has been disrupted almost as much as in Fig. 3. The cell walls are very strong. × 430.

walls are intact. Most of the protein network has dispersed in the acid and the remainder has folded back around the cellulose walls; also, the starch has been freed. The starch was easily separated from the protein giving a starch of moderately low nitrogen content.

When the new corn was steeped along with the old corn of lower moisture, no structural differences could be detected until after the 24th hour. After that time the protein matrix of the new corn broke

up much more rapidly, and by the 36th hour this corn was steeped as well as the old corn after 50 hours (Fig. 4). This was not caused by a difference in moisture content of the grain. Fig. 5 shows the moisture content of old and new corn during the steeping. The old corn had 14.0% moisture, while the new corn had 22.2%, but after 15

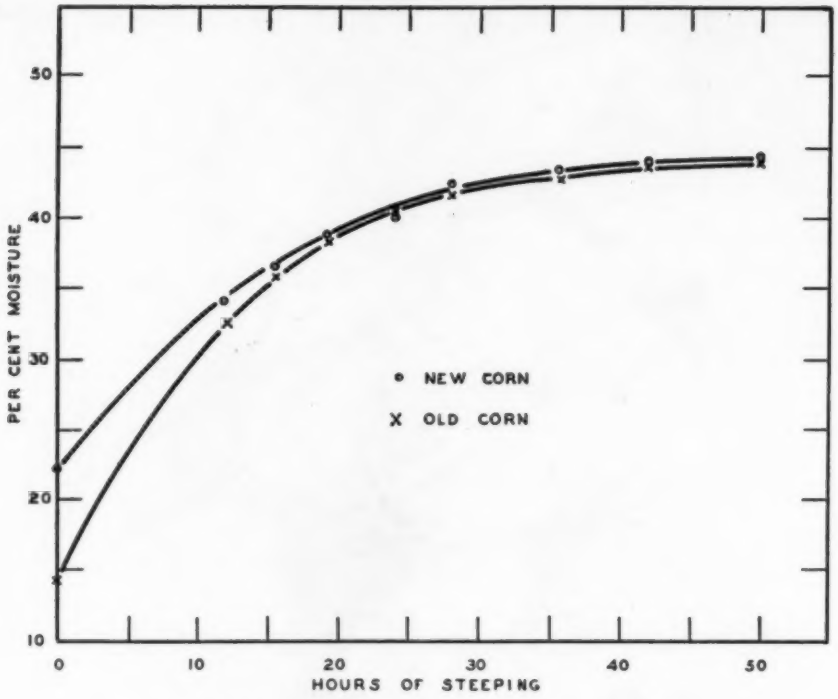


Fig. 5. Moisture absorption of old and new corn during steeping.

to 20 hours both were at relatively the same moisture content. During the rest of the steeping there was no appreciable difference between the two in moisture content. Thus moisture absorption is not a satisfactory criterion for steeping of corn. Continued steeping of the new corn loosens the protein from the cellulose cell walls leaving the cell sections almost free of protein (Fig. 6). This section was quite sturdy, and good ones were easy to obtain. The starch obtained from the new corn steeped for this length of time was higher in nitrogen than the average starch obtained.

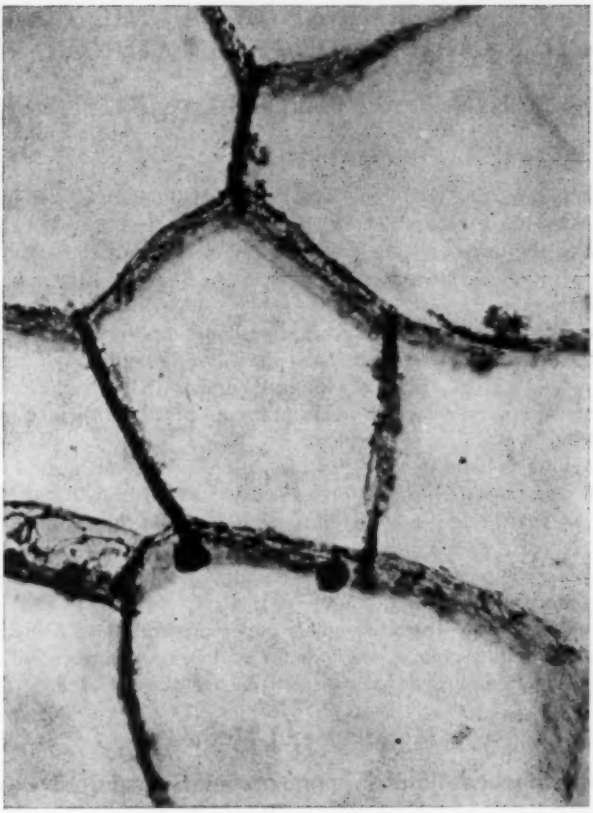

Fig. 6. New corn steeped 50 hours. This section is from the same area as Figs. 1, 2, 3, and 4. The protein matrix has been completely disrupted and a considerable amount has been washed away from the cell walls. Section is 40μ thick. ×430.

## Acknowledgment

The author expresses his appreciation to Dr. B. M. Smitz of Kansas State College and Dr. M. M. MacMasters, and M. Cox of the Northern Regional Research Laboratories, for their suggestions upon this work.

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# A SIMPLIFIED DETERMINATION OF THE COLD WATER ABSORPTIVE CAPACITY OF PREGELATINIZED FLOURS[1]

LEO KLEE, LOUIS SAIR, and LLOYD A. HALL[2]

**ABSTRACT**

A simple, routine technique based on the volume of centrifuged flour-water pastes has been applied in the evaluation of cold water absorptive capacity of unprocessed and pregelatinized wheat and corn flours. This technique is more accurate than an older method based on the measurement of flow properties.

A simple, rapid method for evaluating flours used until recently in this laboratory involved a determination of the water absorption required to produce a paste of a given viscosity as judged empirically by its flow properties. This method, however, lacked precision because of the difficulty in judging the degree of flowability of the paste

A swelling volume method has been used for the evaluation of starch pastes in research laboratories for several years. Brimhall and Hixon (1) have shown that there is a close relationship between the volume of swollen granules of corn starch paste and relative viscosity. Accordingly, in an effort to improve on the rather crude pasting method, the swelling volume principle was applied in a technique particularly designed to evaluate pregelatinized flours and starches. This paper presents data comparing the pasting and swelling-volume methods for evaluation nonprocessed and pregelatinized flours and gives the comparative accuracy of each.

## Materials and Methods

In tests with wheat flour only one pregelatinized flour was available. Blends of this flour in varying proportions with an unprocessed wheat flour were evaluated for cold water absorption by the pasting method and by the swelling-volume procedure. An unprocessed white corn flour, an unprocessed yellow corn flour, and three processed corn flours were also tested by both procedures.

The pasting method involved the addition of sufficient water at laboratory temperature to one ounce of flour contained in a 400-ml. beaker. The flour-water paste is stirred by hand with a glass stirring rod $\frac{3}{16}$ inch in diameter to produce a uniform, slowly flowing paste obtained with a time interval of 10 minutes. The absorption number of the flour was calculated on the basis of flour containing 12.5% moisture, and is determined by multiplying the ounces of water used by 100 when the flour weight is one ounce.

In the swelling volume technique 35 ml. of distilled water at 20 C.$\pm$2 were added with stirring to 3.5 g. flour contained in a 40-ml. reinforced Pyrex centrifuge tube. The flour-water mixture was stirred

[1] Manuscript received October 3, 1947.
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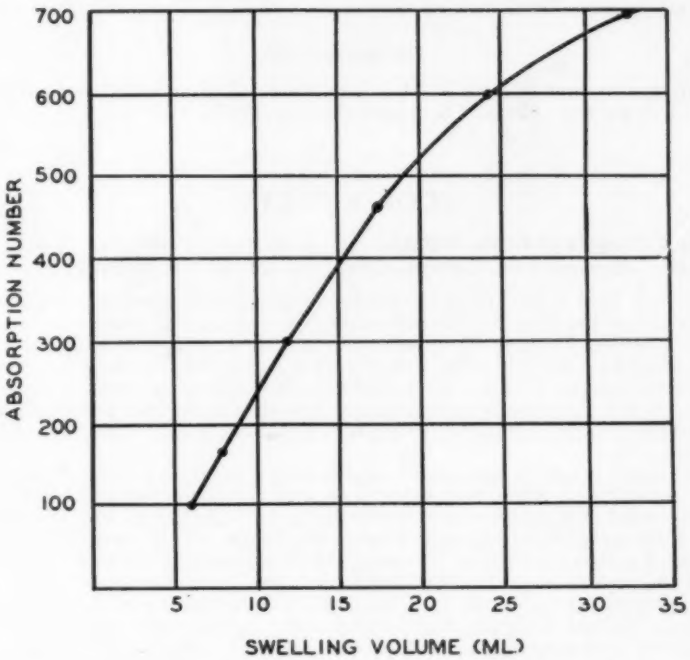
for 3 minutes with a $\frac{3}{16}$-inch glass rod equipped with a rubber tip and then centrifuged for 10 minutes in an International Centrifuge, size 1, Type C, at a speed of 1,300 r.p.m. The resulting volume occupied by the flour is termed "swelling volume."

In preliminary experiments with the swelling volume technique, it was found that stirring the flour-water mixture for 1 and 3 minutes yielded similar results. Allowing the suspensions of unprocessed flours and their blends to stand for periods varying from 4 to 15 minutes before centrifuging had no material effect on swelling volume. In experiments in which the time of centrifuging was varied, the same relative ranking of pregelatinized flour blends was obtained whether 5 or 15 minutes was employed, although the former time yielded higher values.

## Results

A comparison of the data for pasting and swelling volume methods when applied to a number of wheat flours is shown graphically in Fig. 1.




The swelling volume method is lineally related to the water absorption number of the pasting method up to a value of about 500, above which the value for swelling increases more rapidly, resulting in a curvilinear relation.

The data in Fig. 1 were obtained by each of three operators. The experimental error of the swelling volume procedure expressed as a percent of the mean did not exceed 8%, whereas the variability in the pasting procedure was as high as 30%.

TABLE I

PASTING METHOD VERSUS SWELLING VOLUME METHOD IN CORN FLOURS

| Flour used | Pasting method—water absorption number | Swelling volume method—swelling volume, ml. |
|---|---|---|
| Unprocessed white corn flour | 130 | 6 |
| Unprocessed yellow corn flour | 145 | 6 |
| Processed corn flours | | |
| No. 1 | 280 | 10.5 |
| No. 2 | 335 | 12.5 |
| No. 3 | 360 | 13.5 |

The pasting and swelling volume techniques were applied also to the determination of the cold water absorption capacity of corn flours, the data for which are given in Table I. The swelling volume procedure obviously ranks the flours in the same order as the pasting method.

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## BOOK REVIEWS

**Scientific Principles of Grain Storage.** By T. A. Oxley. 103 pp. Northern Publishing Company, Ltd., Liverpool, England. 1948. Price $3.00.

This little book is the first in the English language which presents in simple terminology a few of the scientific fundamentals underlying good grain storage practice. Within its limited scope, it proves abundantly that a number of problems in grain storage, which have heretofore been dealt with in an empirical manner, are best solved by a rational approach based on recognized principles of physics, chemistry, and biology. It will therefore be welcomed not only by the elevator superintendent as a useful reference manual, but also by the cereal chemist who is interested in basic information.

The English workers have made notable strides in clarifying the physics of grain storage, including such factors as heat conduction and convection and moisture translocation as related to temperature differential. Most American workers in the field have been stressing the biological and biochemical aspects of the problem. This book should be of assistance to these investigators in interpreting their biological data in terms of the physical aspects of the problem.

The subject matter of the 13 brief chapters in the book is as follows: 1, the hazards of grain storage; 2, the physics of a grain bulk; 3, water relations of cereal grains; 4, ventilation of storage places; 5, forced ventilation of bulk grain; 6, drying grain for safe storage; 7, the spontaneous heating of stored grain; 8, methods for measuring temperatures in stored grain; 9, measurement of carbon dioxide and water vapor in the intergranular atmosphere; 10, measurement of insect infestation by carbon dioxide production; 11, insect infestations in bulk grain; 12, the more important grain insects; 13, mites as pests of stored grain. References to the literature are given, but the list is not extensive and is principally in the major field of interest of the author.

The author has made a laudable attempt to demolish several of the many unscientific or experimentally unfounded beliefs which abound in this field. A good ex-

ample of this is his insistence that moisture condensation on grain is unnecessary to promote spoilage, since this may be accomplished simply by an increase in the moisture vapor content in the interseed atmosphere. He also refutes the assumption that bin walls must be permeable to air, and that fresh air is desirable for maximum storage longevity of grain. Where little experimental evidence exists, the author's shrewd observations as well as his scientific objectivity make his conclusions very convincing and his theories excellent hypotheses for the guidance of future research. For example, his theory that the duration of grain viability in storage is governed by its initial content of some unknown factor, which is dissipated at a rate depending upon the intensity of the grain respiration, is indeed attractive. The discussion of the relationship of temperature differential in grain bulks to water vapor movement is a lucid exposition of this very basic phenomenon. The suggestion that measurements of interseed humidity may be useful in predicting incipient deterioration is in line with the conclusions of other workers in the field who believe that the interseed humidity rather than moisture content of the grain initiates and controls deterioration due to fungi.

The discussion of the principles of grain drying focuses attention on the advantages of intermittent drying, which process has recently been developed practically in Europe. The author rightly stresses the scarcity of existing knowledge concerning the influence of various drying conditions and techniques on the qualities of wheat.

Much of the material presented is based on experimentation by the author and his co-workers and it is understandable that his own conclusions and experience are the most thoroughly treated. Frequently only a cursory evaluation of other work is made. For example, the author stresses the importance of a well-developed but unidentified subepidermal mold mycelium in sound wheat which he believes to be responsible for the major portion of grain respiration. Other workers have discounted the importance of this previously developed mycelium both in the respiratory process, and as a factor in storage deterioration and have stressed instead the proliferation under, favorable conditions, of a mycelium from mold spores of other species which exist beneath the seed coat. The data of the author, which show an increase in the respiration of damp wheat with time following harvest, would appear to better support the latter hypothesis.

The first chapter discusses storage hazards in too cursory a manner. A description of deteriorative processes in grain refers to heating as though it preceded molding, sprouting, and rotting. This is incorrect since heating usually follows or accompanies rather than precedes the activity of molds, insects, and biological processes. The listing of means for recognition of deterioration omits the very important factors of odor and discoloration of grain due to heat damage.

In the discussion of hygroscopic moisture equilibria of wheat, it is unfortunate that among the various publications in the field which were cited, no reference was made to the well-known paper of Coleman and Fellows (Cereal Chem. **2**: 275–287, 1925) which is accepted most widely by American workers as the authority on cereal grain hygroscopicity. The author suggests that hygroscopic equilibrium determinations for wheat might be more valid if measurements were made of intergranular air humidities at equilibrium, after the grain has been brought to various moisture levels. It should be pointed out that such measurements have been made although not widely publicized (J. E. Anderson, AOM Bull., pp. 707–710, 1936, and unpublished data) and they have yielded air humidity data at equilibrium approximately 10% lower at corresponding moisture values than the results obtained by the hygroscopic equilibrium methods. This apparent discrepancy awaits an explanation.

In his discussion of the probable advantages of airtight storage of grain, the author might well have pointed out that no authentic information exists concerning the influence of elevated carbon dioxide concentrations, such as might arise in sealed storage, on the quality of wheat.

The chapter dealing with insects prevalent in grain describes these pests and their habits concisely. The inclusion of illustrations would have increased the practical utility of this chapter. A more notable lack, however, is the complete absence of any discussion concerning fumigants.

This book will prove of value to individuals connected with the buying, storage, shipping, and processing of cereal grains.

MAX MILNER
Department of Milling Industry
Kansas State College
Manhattan, Kansas

**Isomerism and Isomerisation.** By Ernst David Bergmann. 138 pp. Interscience Publishers, Inc., New York. 1948. Price $3.50.

This little booklet is an expansion of six lectures given under the general heading of the progress of chemistry. It deals with six subjects of importance to the organic chemist, namely, (I) resonance phenomena in organic molecules, (II) cis-trans isomerism and cis-trans interconversion, (III) isomerisation of olefinic structures, (IV) mechanism of substitution reactions; racemisation and Walden inversion, (V) isomerisation of paraffins and related phenomena, and (VI) mechanism of intramolecular rearrangements.

It is a very interesting and well-written book which brings the reader to the forefront of research in these particular fields of organic chemistry and the theories involved therein. The book contains an excellent literature survey for the specialist and it demonstrates in a striking manner the excellent results which are derivable from the application of physical methods to organic chemistry.

The book was written not with the object of covering the whole field but with the intention of stimulating thought and discussion. This it certainly accomplishes and the reviewer can recommend it both to research students and teachers.

F. SMITH
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St. Paul, Minnesota

**Acetylene Chemistry.** By Ernst David Bergmann. 108 pp. Interscience Publishers, Inc., New York. 1948. Price $3.00

This small book like the previous one, *Isomerism and Isomerisation of Organic Compounds*, by the same author is based upon lectures given at seminars dealing with progress in chemistry. This one might well have been called recent advances in the chemistry of acetylene and its compounds.

The book has been written by one well acquainted with organic chemistry and it will prove valuable both to those interested in the general field of organic chemistry and the specialists in acetylene chemistry. For the specialists there is a good literature survey. The progress in acetylene chemistry set forth illustrates in a very striking manner the versatile nature of acetylene and its derivatives which still remains to be exploited.

The first of its three chapters deals with the classification of the reactions of acetylene and the relation between the molecular structure of acetylene compounds and their physical properties; the second discusses the addition reactions of acetylene with ketonic substances; and the third deals with the formation and properties of vinyl- and divinyl-acetylenes and the chemistry of cyclo-octatetraene. The reviewer noted with interest the application and interpretation of the results of physical measurements of acetylene compounds in connection with structural studies.

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**Synthetic Methods of Organic Chemistry. A Thesaurus.** Volume I. 1942–1944. By W. Theilheimer. 254 pp. Interscience Publishers, Inc., New York and London. 1948. Price $5.00.

This first volume provides up-to-date methods in organic chemistry which appeared in the literature during the years 1942–1944, inclusive. For each method the number of steps involved and also the yields are quoted. It is pleasing to find that these methods are arranged according to the fundamental reactions of organic chemistry (the way in which organic chemistry is best taught), and not solely according to trivial names or authors' names. The methods are also helpfully subdivided according to the reagents employed and further aid is provided by the alphabetical index which includes names of methods and types of compounds.

The book is intended to serve as a preliminary reference book for laboratory use. Details of the methods can be obtained from the original literature to which reference is made. This book is an extension of Houben's book on methods and Weygand's Organisch-chemische Experimentierkunst. New reactions or variations of old ones not to be found in Houben are given either in this volume or will be recorded in the volumes to follow. When the series is brought up-to-date, it will provide an invaluable tool not only to those whose studies were interrupted by the war but to all those engaged in research in organic chemistry.

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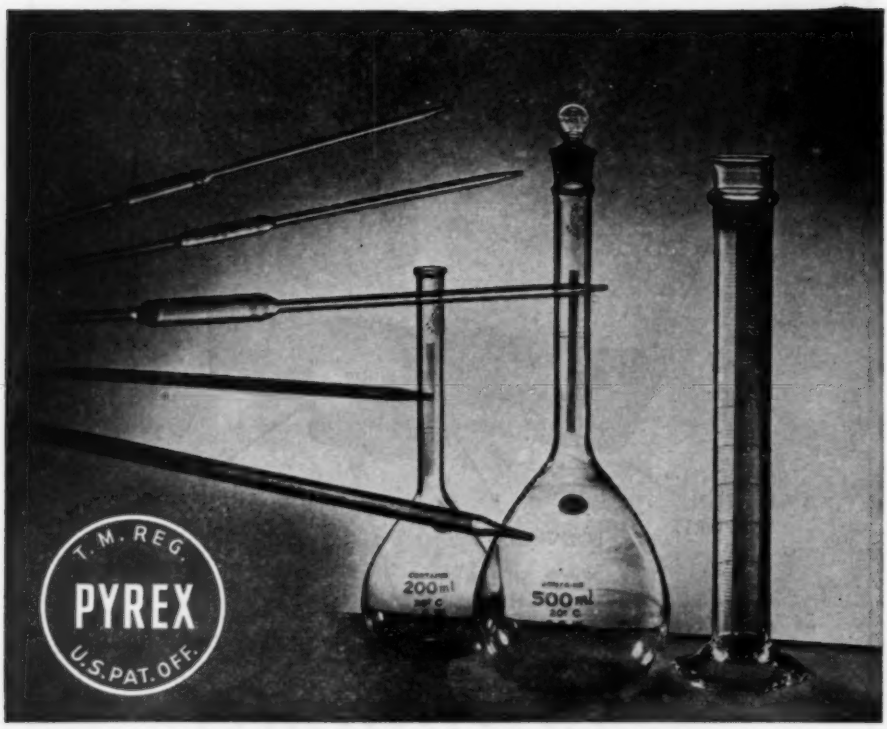


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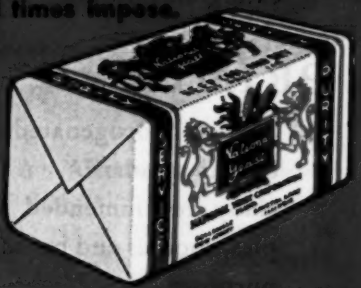

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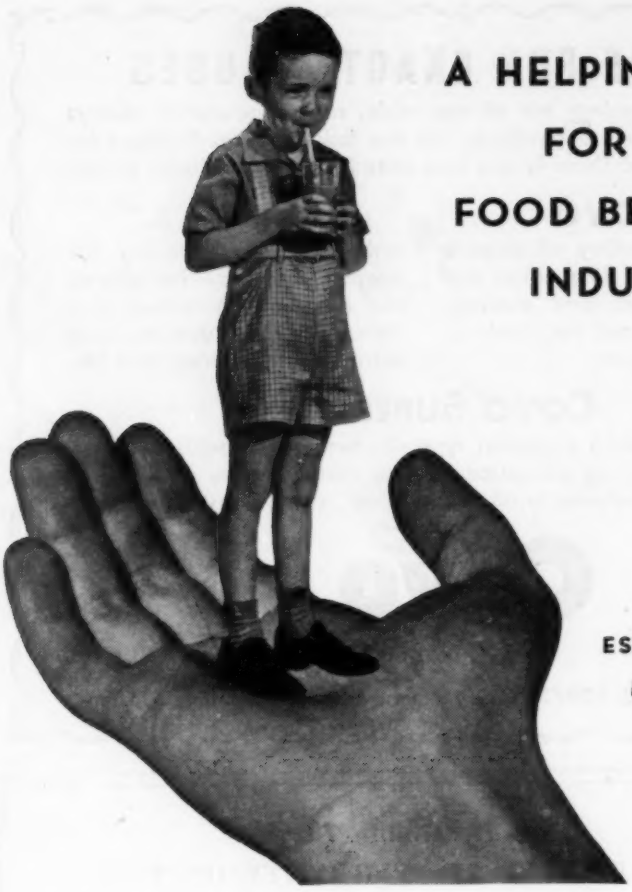

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